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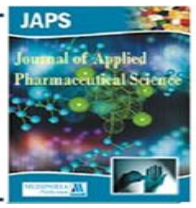

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Nanoparticle: An overview of preparation and characterization

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ABSTRACT

In recent years, there has been an exponential interest in the development of novel drug delivery systems using nanoparticles. Nanoparticles can offer significant advantages over the conventional drug delivery in terms of high stability, high specificity, high drug carrying capacity, ability for controlled release, possibility to use in different route of administration and the capability to deliver both hydrophilic and hydrophobic drug molecules. This review focuses on classification, methods of preparation, characterisation, application, advantages of nanoparticles and health perspectives.

Key words: Nanoparticles, Preparation, Characterization, Applications.

INTRODUCTION

The prefix “nano” has found in last decade an ever-increasing application to different fields of the knowledge. Nanoscience, nanotechnology, nanomaterials or nanochemistry are only a few of the new nano-containing terms that occur frequently in scientific reports, in popular books as well as in newspapers and that have become familiar to a wide public, even of non-experts. 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 10^9 times. So, the nanosized 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 is the science of the small; the very small. It is the use and manipulation of matter at a tiny scale. At this size, atoms and molecules work differently, and provide a variety of surprising and interesting uses. Nanotechnology and Nanoscience studies have emerged rapidly during the past years in a broad range of product domains. It provides opportunities for the development of materials, including those for medical applications, where conventional techniques may reach their limits. Nanotechnology should not be viewed as a single technique that only affects specific areas. Although often referred to as the ‘tiny science’, nanotechnology does not simply mean very small structures and products. Nanoscale features are often incorporated into bulk materials and large surfaces. Nanotechnology represents the design, production and application of materials at atomic, molecular and macromolecular scales, in order to produce new nanosized materials (Hahens et al., 2007). Pharmaceutical nanoparticles are defined as solid, submicron-sized (less than 100 nm in diameter) drug carrier that may or may not be biodegradable. The term nanoparticle is a combined name for both nanosphares and nanocapsules. Nanospheres are matrix system in which drug is uniformly dispersed, while nanocapsules are the system in

which the drug is surrounded by a unique polymeric membrane. This systemic review focuses on Classification, method of preparation, Characterization, application, health prospective and Pharmacological aspects of nanoparticles (Couvreur P et al., 1995).

Classification of nanoparticles

There are various approaches for classification of nanomaterials. Nanoparticles are classified based on one, two and three dimensions **(**Hett, 2004**)**.

One dimension nanoparticles

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

Two dimension nanoparticles

Carbon nanotubes (CNTs):

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, single-walled 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 (Kohler et al., 2004). They display metallic or semi conductive properties, depending on how the carbon leaf is wound on itself. The current density that nanotubes can carry is extremely high and can reach one billion amperes per square meter making it a superconductor. The mechanical strength of carbon nanotubes is sixty times greater than the best steels. Carbon nanotubes have a great capacity for molecular absorption and offering a three dimensional configuration. Moreover they are chemically and chemically very stable.

Three dimension nanoparticles

Fullerenes (Carbon 60):

Fullerenes are spherical cages containing from 28 to more than 100 carbon atoms, contain C_{60}. This is a hollow ball composed of interconnected carbon pentagons and hexagons, resembling a soccer ball. Fullerenes are class of materials displaying unique physical properties. They can be subjected to extreme pressure and regain their original shape when the pressure is released. These molecules do not combine with each other, thus giving them major potential for application as lubricants. They have interesting electrical properties and it has been suggested to use them in the electronic field, ranging from data storage to production of solar cells. Fullerenes are offering potential application in the rich area of nanoelectronics. Since fullerenes are empty structures with dimensions similar to several biological active molecules, they can be filled with different substances and find potential medical application (Tomalia, 2004).

Dendrimers:

Dendrimers represents a new class of controlled-structure polymers with nanometric dimensions. Dendrimers used in drug delivery and imaging are usually 10 to 100 nm in diameter with multiple functional groups on their surface, rendering them ideal carriers for targeted drug delivery (Wiener et al., 1994). The structure and function of dendrimers has been well studied. Contemporary dendrimers can be highly specialized, encapsulating functional molecules (i.e., therapeutic or diagnostic agents) inside their core (Li et al., 2007). They are considered to be basic elements for large-scale synthesis of organic and inorganic nanostructures with dimensions of 1 to 100 nm (Tomalia et al., 2004). They are compatible with organic structure such as DNA and can also be fabricated to metallic nanostructure and nanotubes or to possess an encapsulation capacity (Fu et al., 2007). Dendrimers have different reactive surface groupings (nanostructure) and compatible with organic structure such as DNA so their prolific use is particularly in the medical and biomedical fields.The pharmaceutical applications of dendrimers include nonsteroidal anti-inflammatory formulations, antimicrobial and antiviral drugs, anticancer agents, pro-drugs, and screening agents for high-throughput drug discovery(Cheng Y et al., 2008). Dendrimers may be toxic because of their ability to disrupt cell membranes as a result of a positive charge on their surface (Mecke et al., 2004).

Quantum Dots (QDs):

Quantum dots are small devices that contain a tiny droplet of free electrons.QDs are colloidal semiconductor nanocrystals ranging from 2 to 10 nm in diameter. QDs can be synthesized from various types of semiconductor materials via colloidal synthesis or electrochemistry. The most commonly used QDs are cadmium selenide (CdSe), cadmium telluride (CdTe), indium phosphide (InP), and indium arsenide (InAs).

Quantum dots can have anything from a single electron to a collection of several thousands. The size, shape and number of electrons can be precisely controlled. They have been developed in a form of semiconductors, insulators, metals, magnetic materials or metallic oxides. It can be used for optical and optoelectronic devices, quantum computing, and information storage. Colour coded quantum dots are used for fast DNA Testing. Quantum ots (QDs) refer to the quantum confinement of electrons and hole carriers at dimensions smaller than the Bohr radiuos. QD nanocrystals are generally composed of atoms from groups II and VI (that is CdSe, CdS, and CdTe) or II and V (such as In P) at their core. A shell (that is ZnS and CdS) can be further introduce to prevent the surface quenching of excitons in the emissive core and hence increase the photostability and quantum yield of emission (Goldberg M et al., 2007). QDs also provide enough surface area to attach therapeutic agents for simultaneous drug delivery and in vivo imaging, as well as for tissue engineering (Larson DR et al., 2003).

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:

Emulsion-Solvent Evaporation Method:

This is one of the most frequently used methods for the preparation of nanoparticles. Emulsification-solvent evaporation 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 (Song et al., 1997**).** Modification of this method is known as high-pressure emulsification and solvent evaporation method (Jaiswal et al., 2004**).** This method involves preparation of a emulsion which is then subjected to homogenization under high pressure followed by overall stirring to remove organic solvent (Soppinath et al., 2001)**.**The size can be controlled by adjusting the stirring rate, type and amount of dispersing agent, viscosity of organic and aqueous phases and temperature (Tice et al., 1985**).** However this method can be applied to liposoluble drugs and limitation are imposed by the scale up issue. Polymers used in this method are PLA (Ueda et al., 1997**),** PLGA (Tabata et al., 1989**),** EC (Bodmeier et al., 1990**),** cellulose acetate phthalate (Allemann et al., 1993**),** Poly (ε-caprolactone)\ (PCL) (Lemarchand et al., 2006**),** Poly (β-hydroxybutyrate) (PHB) (Koosha et al., 1989**).**

Double Emulsion and Evaporation Method:

The emulsion and evaporation method suffer from the limitation of poor entrapment of hydrophilic drugs .Therefore 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 (Vandervoort et al., 2002).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 (Ubrich et al., 2004).

Salting Out Method:

Salting out based on the separation of a water-miscible solvent from aqueous solution via a salting-out effect (Catarina PR et al., 2006). Salting-out 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 (Allemann et al., 1993).This technique used in the preparation of PLA, Poly(methacrylic) acids, and Ethyl cellulose nanospheres leads to high efficiency and is easily scaled up (Quintanar-Guerrero et al., 1998) (Jung et al., 2000). Salting out does not require an increase of temperature and therefore may be useful when heat sensitive substances have to be processed (Lambert et al., 2001).The greatest disadvantages are exclusive application to lipophilic drug and the extensive nanoparticles washing steps**.**

Emulsions- Diffusion Method:

This is another widely used method to prepare nanoparticles. 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. Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of nanospheres or nanocapsules, according to the oil-to-polymer ratio. Finally, 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. Disadvantages are the high volumes of water to be eliminated from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase during emulsification, reducing encapsulation efficiency (Takeuchi et al., 2001).Several drug- loaded nano particles were produced by the technique, including mesotetra (hydroxyphenyl) porphyrin-loaded PLGA (p-THPP) nano particles (Vargas et al., 2004), doxorubicin-loaded PLGA nano particles (Yoo et al., 1999), and cyclosporine (cy-A-); loaded sodium glycolate nanoparticles (El-shabouri, 2002).

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 semipolar 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.

The solvent is then removed from the suspensions under reduced pressure. The rates of addition of the organic phase into the aqueous phase affect the particles size. It was observed that a decrease in both particles size and drug entrapment occurs as the rate of mixing of the two phase increases (Fessi et al., 1989). Nano precipitation method is well suited for most of the poorly soluble drugs.

Nanosphere size, drug release and yield were shown to be effectively controlled by adjusting preparation parameters. Adjusting polymer concentration in the organic phase was found to be useful in the production of smaller sized nanospheres through restricted to a limited range of the polymer to drug ratio (Chorney et al., 2002).

Characterization of Nanoparticles

Nanoparticles are generally characterized by their size, morphology and surface charge, using such advanced microscopic techniques as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). 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

Particle size:

Particle size distribution and morphology are the most important parameters of characterization of nanoparticles. Morphology and size are measured by electron microscopy. The major application of nanoparticles is in drug release and drug targeting. 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 (Redhead et al., 2001). 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 (Betancor et al., 2000).

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 (DeAssis et al., 2008).

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 (Jores et al., 2004). 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 (Molpeceres et al., 2000).

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 (Molpeceres et al., 2000).

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 (Muhlen et al., 1996). Instrument provides a topographical map of sample based on forces between the tip and the sample surface. Samples are usually scanned in contact or non-contact 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 (Polakovic et al., 1999).

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 (Pangi et al., 2003).

Surface hydrophobicity:

Surface hydrophobicity can be determined by several techniques such as hydrophobic interaction chromatography, biphasic partitioning, adsorption of probes, contact angle measurements etc. Recently, several sophisticated analytical techniques are reported in literature for surface analysis of nanoparticles. X – ray photon correlation spectroscopy permits the identification of specific chemical groups on the surface of nanoparticles (Scholes et al., 1999).

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. In order to obtain such information most release methods require that the drug and its delivery vehicle be separated. The drug loading of the nanoparticles is generally defined as the amount of drug bound per mass of polymer (usually moles of drug per mg polymer or mg drug per mg 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 ultracentrifugation, ultra filtration, gel filtration, or centrifugal ultrafiltration. Quantification is performed with the UV spectroscopy or HPLC. 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 (Kreuter, 1983) (Magenhein et al., 1993).

Application of Nanoparticles (Pangi et al., 2003)

Application of nanotechnology in the different field is summarised in table 1.

Table 1: Application of nanotechnology in the different field.

Applied field	Application
Nanomedicines	Nano drugs, Medical devices, Tissue engineering
Chemical and Cosmetics	Nanoscale chemicals and compounds, paints, coatings etc
Materials	Nanoparticles, carbon nanotubes, biopolymers, points, coatings
Food Sciences Environment and Energy	Processing, nutracetical food, nanocapsules. Water and air purification filters, fuel cells, photovoltaic
Military and Energy	Biosensors, weapons, sensory enhancement
Electronics	Semiconductors chips, memory storage, photonica, optoelectronics
Scientific Tools	Atomic force, microscopic and scanning tunnelling microscope
Agriculture	Atomic force, microscopic and scanning tunnelling microscope.

Health Implication of Nanoparticles

It is important to differentiate between ‘free’ and ‘fixed’ nano particles. The formers pose a direct health threat because they are more difficult to contain due to airborne and can be inhaled. Nanoparticles can enter 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 (Hoet et al., 2004).

Lungs: Based on three particle types titanium dioxide (TiO_2) carbon black and the diesel particles, hazards studies in rats, demonstrate that ultrafine nanoparticles administration to the lung produce more potent adverse effect in the form of inflammation and subsequent tumors compared with larger sized particles, of identical chemical composition at equivalent mass concentration. Surface properties such as surface chemistry may play a significant role in nanoparticles toxicity (Lee et al., 1998) .

Intestinal Tract: The epithelium of the small and large intestinal is in close contact with ingested material so that nutrients can be utilized. A mixture of disaccharides, peptides, fatty acids and monoglycerides generated but digestion in small intestine are further transformed and taken in the villi.

Charged particles like carboxylated polystyrene nano particles or those composed of positively charged polymer exhibit poor oral bioavailability through electrostatic repulsions and means entrapment (Jani et al., 1989). The smaller the particles diameter the faster they could penetrate the mucus to reach the colonic eutrocytes;14nm diameter permeated within 2 mints, 415 nm

particles look 30 mints while 1000 nm particles were unable to translocate this barrier (Jani et al., 1990).

Skin: Particles 500-1000 nm in size theoretically beyond the realms of nano technology can penetrate and reach the lower levels of human skin, 128 and smaller particles are likely to more deeper into the skin (Lademann et al., 1989).

Advantages of Nanoparticles

Significant advantages of nanoparticles are given in table 2.

Table 2: Advantages of nanoparticles.

Increased bioavailability
Dose proportionality.
Smaller dose form.
Increased surface area results in a faster dissolution of the active agent in an aqueous environment, such as the human body. 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. These are many technical, challenges in developing the following techniques:- Virus-like systems for intracellular systems, Architecting of biomimetic polymers, control of sensitive drugs, functions (of active drug targeting, bioresponsive triggered systems, systems interacting with me body smart elivery), nanochips for nanoparticle release, carriers for advanced polymers for the delivery of therapeutic peptide / proteins. Drug delivery techniques were established to deliver or control the amount & rate. Most major and established internal research programmes on drug delivery that are formulations and dispersion containing components down to nano sizes.

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 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. 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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