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Review article

Metal nanoparticles: Synthesis, characterization, toxicity and regulatory aspects

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Abstract

Metal nanoparticles have emerged as a prospective mode of drug delivery. They can be used to delivery various classes of drugs into the systemic circulation. Moreover, it can also be used in targeting cytotoxic drugs to cancer sites which is a great advantage. The present article details about methods of synthesis of various types of metal nanoparticles and their methods of characterizations. It also brings to light about the causes and implications of toxic effects posed by these nanoparticles. Toxicity of these metal nanoparticles has become an issue of growing concern which is a global threat. The article summarizes these toxicity issues governing various types of nanoparticles and their regulatory basis. The toxicity and regulatory issues are some hurdles which need to be addressed in order to make these nanoparticles a widely complaint mode of drug delivery.

Keywords: Metal nanoparticles, drug delivers.

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1. Introduction

Nanotechnology is the most promising technology that can be applied to almost all spheres of life, ranging from electronic storage systems, pharmaceutical, biotechnology [1], magnetic separation [2] magnetic separation and pre-concentration of target analytes, targeted drug delivery, [3,4] and vehicles for gene and drug delivery [1,3-5], defense, transportations heat transfer to sports and aesthetics.In nanotechnology, a particle is defined as a small object that behaves as a whole unit in terms of its transport and properties. Nanoparticles are sized between 1 and 100 nanometers and may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials [6, 7].

There are basically two types of nanoparticles:

1.1. Engineered Nanoparticles: “NPs,” with diameters of < 100 nm, are devices and systems intentionally designed and created with chemical and/or physical processes having specific properties [8,9]and to meet specific applications. They can be end products in and of themselves, as in the case of quantum dots or pharmaceutical drugs, or they can be components later incorporated into separate end products, such as carbon black in rubber products. Either way, the particle’s physical properties are extremely important to their performance and the performance of any product into which they are ultimately incorporated [9].

1.2 Nonengineered nanoparticles: on the other hand, are unintentionally generated or naturally produced, such as atmospheric nanoparticles created during combustion. With nonengineered nanoparticles, physical properties also play an important role as they

determine whether or not ill effects will occur as a result of the presence of these particles [9]. Transition metal oxides are used in catalysis [10], magnetocooling [11], optical and recording devices [12-13], purification of enzymes and other biological materials [14], water purification devices [15], magnetic field assisted radionuclide therapy [16], embolics [17-19], and targeted drug delivery [20]. Among the transition metal oxides, titanium dioxide (TiO2), cupric oxide (CuO), and zinc oxide (ZnO) have received the most attention due to their unique physical and chemical properties. Some NP's are used in Nanocatalysis. Nanoparticles show striking novel catalytic properties including greatly enhanced reactivities and selectivities as compared to their bulk counterparts [21]. Nanocatalysis has emerged as a field at the interface between homogeneous and heterogeneous catalysis and offers unique solutions to the demanding requirements for catalyst improvement. Alloy nanoparticles show different structural and physical properties than bulk samples [22-23]. Increase in solid solubility of alloy components with decreasing particle size is one of the prominent effects. Bimetallic nanoparticles (BMNP) have excelled monometallic nanocrystals owing to their improved electronic, optical and catalytic performances [24-25].

BMNP often improve the selectivity of metal catalyzed reactions. NP's are most commonly used in Drug delivery due to their small size they can penetrate physiological barriers, and travel in the circulatory system of a host. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly ethylene glycol (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes [26-29]. NP's are used in the drug delivery system because its particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration, they control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects, site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance and system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.

NP's are used in many areas in our industry but they do have some limitations i.e.

1. Due to small size and large surface area particle aggregation may occur, making physical handling of nanoparticles difficult in liquid and dry forms.
2. Small particles size and large surface area readily result in limited drug loading and burst release.

2. Synthesis of nanoparticles:

Nanoparticles can be synthesized chemically, physically or biologically. Production of nanoparticles can be achieved through different methods. Most common approaches include Solid state methods, Vapour methods, Chemical synthesis / Solution methods and Gas-phase synthesis methods [16].

2.1 Direct method: Typically, metal atoms are formed by reduction (1) or decomposition of the metal precursor (2A). Free atoms are highly unstable and nucleate (3) quickly, until this process is prevented by a stabilizer (4). The reducing agent may also act as a stabilizer [30]. This is called the direct method of synthesis.

A major advantage of the direct method is its simplicity. However, it cannot always be applied, as only few metal precursors meet the above-mentioned requirements. In case of difficulties in preparing nanoparticles directly in ionic liquids, the following alternative approach (indirect method) may be considered.

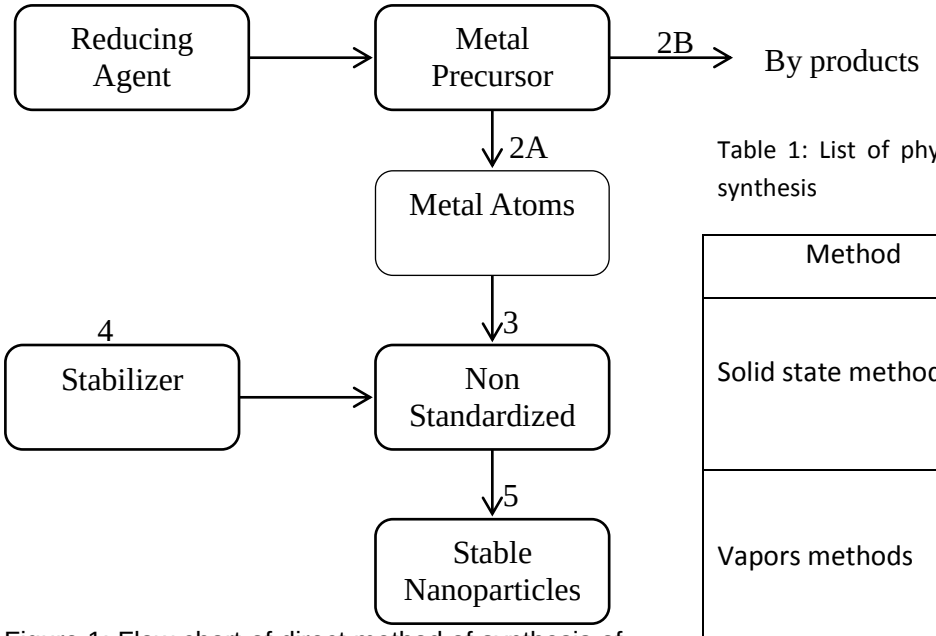

Figure 1: Flow chart of direct method of synthesis of nanoparticles

2.2 Indirect method: This is for the synthesis of nanoparticles in ionic liquids: After dissolution (not always needed) of the metal precursor in an organic solvent, the former is reduced in the presence of an external stabilizer, then the ionic liquid (IL) is added and solvent (and any by-products) are removed. Note that the stabilizer has to be well soluble in the solvent in order to obtain a good dispersion of thus prepared nanoparticles [31].

Table 1: List of physical methods used in nanoparticle synthesis

Method	Types
Solid state methods	Grinding Milling Mechanical alloying techniques
Vapors methods	Physical vapour deposition Chemical vapour deposition
Gas phase synthesis methods	Flame pyrolysis Electro-explosion Laser ablation Plasma synthesis techniques

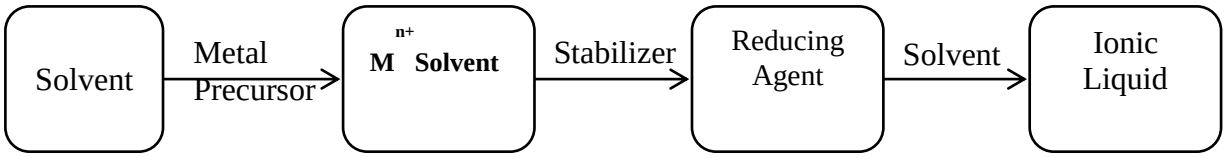

Figure 2: Flow chart of indirect method of synthesis of nanoparticles

These can be further classified as:

2.3 Physical methods: Nanoparticles can be prepared via a combination of “top-down” or the physical methods and “bottom-up” or the chemical methods. First, free metal atoms are obtained via a chosen physical method and their subsequent agglomeration is controlled by a stabilizer. Below we give a short overview of a physical method, which found attention in the past or recent literature [31].

2.4 Chemical synthesis/solution methods: Chemical approaches are the most popular methods for the production of nanoparticles. In general, certain technical barriers (as opposed to e.g. price or environmental barriers) can be pointed out as the main drawback for their success. In general, additional processes need to be carried out to prevent agglomeration.

2.4.1 Sol-gel approach: There are difficulties in simultaneously controlling all parameters. For this reason, reproducibility is often an issue. In general, low yields are obtained by

using the solgel approach. However, upscaling capabilities are expected to resolve the problem. Agglomeration is also a concern when using sol-gel approaches. To finalise, there are minor environmental problems, such as large volumes of contaminated solvent (usually water) to deal with. Closed-loop processes might help to solve this problem [16].

2.4.2 Colloidal chemistry: Environmental problems identified for the sol-gel approach are also applicable to this method. According to some sources, colloidal chemistry methods will continue to be developed over the next 5-10 years. Other precipitation processes include hydrothermal methods [16].

A wide range of nanoparticles can be produced using physical methods with little modfication for different metals, but the main disadvantages of these methods are the quality of the product, which is less as compared to nanoparticles produced by chemical methods. Usually these methods require costly vacuum systems or equipments to prepare nanoparticles (plasmas).

2.5 Other novel methods [16]:

1. **Microwaves techniques**: are expensive processes due to energy conversion inefficiencies.
2. **Ultrasound techniques** (which can be used in conjunction with some other techniques) and electrodeposition processes, which main barriers can be considered of a technical nature.
3. **Biological/biomimetic techniques** face also important technical barriers. Better scientific understanding is still needed, but is improving all the time, according to some experts. Some of these techniques promise enormous versatility and are now only in the earliest stages of exploration. Large-scale synthesis would be another major hurdle to be overcome.
4. **Supercritical Fluid (SCFs)** precipitation process is a quite complex process, requiring costly equipment.

3. Types of metal nanopaticles and their synthesis [32-73, 139]:

3.1 Silver: Currently, many methods have been reported for the synthesis of Ag-NPs by using chemical, physical, photochemical and biological routes. Each method has advantages and disadvantages with common problems being costs, scalability, particle sizes and size distribution. Among the existing methods, the chemical methods have been mostly used for production of Ag-NPs [139].

3.1.1 Chemical methods: Chemical methods provide an easy way to synthesize Ag-NPs in solution. For the preparation of silver nanoparticles 300 mg of AgNO3 is added to 180 cm3 of ethanol at a temperature of 60oCwith constant stirring. Maribel and coworkers synthesized silver nanoparticles using two stabilizing agents, sodium dodecyl sulphate (SDS) and sodiumcitrate [32]. Silver nitrate solution (1-6mM) was used as a salt precursor Hydrazine hydrate solution with a concentration of 2.0 - 12 mM and sodium citrate solution (1.0 - 2.0 mM) were used as reducing agents. Sodiumcitrate was also used as stabilizing agent at room temperature. The transparent colorless solution was converted to the characteristic pale yellow color which indicated the formation of silver nanoparticles. These were then purified by centrifugation. For the removal of excess silver ions, the silver colloids were washed thrice with deionized water under nitrogen stream. A dried powder of the nanosize silver was obtained by freeze-drying. Monodisperse samples of silver nanocubes were synthesized in large quantities by reducing silver nitrate with ethylene glycol in the presence of polyvinyl pyrrolidone (PVP) [33], the so-called polyol process. In this case, ethylene glycol served as both reductant and solvent.

3.1.2 Physical methods: For a physical approach, the metallic NPs can be generally synthesized by evaporation–condensation, which could be carried out by using a tube furnace at atmospheric pressure. However, in the case of using a tube furnace at atmospheric pressure there are several drawbacks such as a large space of tube furnace, great consumption energy for raising the environmental temperature around the source material and a lot of time for achieving thermal stability. Therefore, various methods of synthesis of Ag-NPs based on the physical approach have been developed. A thermal-decomposition method was developed to synthesize Ag-NPs in powder form [34]. The Ag-NPs were formed by decomposition of a Ag1+–oleate complex, which was prepared by a reaction with AgNO3 and sodium oleate in a

water solution, at high temperature of 290oC. Average particle size of the Ag-NPs was obtained of about 9.5 nm with a standard deviation of 0.7 nm. This indicates that the Ag-NPs have a very narrow size distribution.

3.2 Copper:

The production of copper nanoparticles is much more challenging in comparison to noble metals because copper nanoparticles are quite sensitive to aqueous solutions. When copper nanoparticles are placed in the open air, aggregation appears immediately due to surface oxidation. To avoid this problem, an inert environment, such as argon or nitrogen [35-36] is used. In some cases, inorganic solvents have been used.

3.2.1 Chemical methods: During the chemical synthesis process of copper nanoparticles, the growth and morphology can be controlled by optimizing reaction conditions, such as surfactant's temperature and concentration, precursor, capping/stabilizing agent and the type of solvent.[37-39] Using these optimum reaction conditions, a narrow size distribution during chemical synthesis can be achieved. These methods for the production of copper nanoparticles are appropriate for laboratory-scale synthesis but are not economical for a large-scale or commercial setup. In the chemical reduction techniques, a copper salt is reduced by a reducing agent such as sodium borohydride, [40-44] hydrazine [39,45,46] ascorbate, [47] polyol, [48] isopropyl alcohol with cetyltrimethylammonium bromide (CTAB), [49] as well as glucose [50].

3.2.2 Physical methods: one of the methods is discussed below

Mechanical or ball milling method: Mechanical mills which are commonly used for the synthesis of copper nanoparticles are planetary, vibratory, uniball and attritor [51]. Due to mechanical limitations, it is very difficult to produce ultrafine particles using these techniques or it takes very long time. However, simple operation, low cost of production of nanoparticles and the possibility to scale it to produce large quantities are the main advantages of mechanical milling [52]. The important factors affecting the quality of the final product are the type of mill, milling speed, container, time, temperature, atmosphere, size and size distribution of the grinding medium, process control agent, weight ratio of ball to powder and extent of filling the vial [53].

3.3 Gold:

3.3.1 Chemical methods: The citrate method which is one of the best-known methods for producing gold nanoparticles that involves reduction of HAuCl4 by sodiumcitrate was first developed by Turkevich et al. [54]. In polyol method AuNP-PEG-Asolution is prepared by mixing 0.2mM aqueous solution of thiolated poly ethylene-glycol (PEG) with AuNP H2Osystem.Asolution of AuNPPEG- B is prepared by mixing 0.2 mM aqueous solution of PEG with AuNP-citrate solution. The method is preferred over other processes because of fewer chances of impurities and role of ethylene glycol as a solvent as will as a reducing agent.

3.3.2 Physical methods:

1. γ-irradiation method: This was proved to be best for the synthesis of gold nanoparticles with controllable size and high purity. The γ-irradiation method is adopted to synthesize gold nanoparticles with size 5 - 40 nm. In this method natural polysaccharide alginate solution was used as stabilizer [55].

2. Photochemical synthetic approach: In this method, HAuCl4 and aqueous glycine solution was exposed to UV irradiation. Basically amino acid capped gold nanoparticles were used as photochemical initiator which is then further functionalized with glycine [56]. A new technique of photochemical method has been reported for the preparation of gold-polyethylene glycol core-shell nanoparticles with size 10 – 50 nm in water by adopting redox and polymerization reactions. In this method gold salt was reduced by radical formation coated with polyethylene glycol diacrylate by UV process with the help of photo-initiator 2-hydroxy-2- methyl-1-phenyl-1-propane [58].

3. Microwave irradiation: Microwave irradiation method was adopted to synthesize gold nanoparticles by using citric acid as a reducing agent and cetyltrimethyl ammonium bromide (CTAB) as a binding agent [57].

3.4 Aluminium:

The techniques for synthesizing aluminum nanoparticles can be divided into solidphase,

liquid-phase and gas-phase processes. The solid-phase techniques include mechanical ballmilling and mechanochemical, the liquid-phase techniques include laser ablation, exploding wire, solution reduction, and decomposition process, whereas the gas-phase processes include gas evaporation, exploding wire, and laser ablation process.

3.4.1 Solid-phase synthesis:
Mechanical ball milling: Mechanical milling as a solid state synthesis usually performed using ball milling equipments that generally divided to “low energy” and “high energy” category based on the value of induced the mechanical energy to the powder Mixture [60]. The objective of milling is to reduce the particle size and blending of particles in new phases. The different type of ball milling can be used for synthesis of nanomaterials in which balls impact upon the powder charge [59].

3.4.2 Liquid-phase synthesis
Laser ablation
Pulsed laser ablative deposition (PLD) is an attractive synthetic method owing to its ability to produce nanoparticles with a narrow size distribution and a low level of impurities[61,62]. Aluminum nanoparticles with diameters of tens to 500 nm of various shapes can be prepared by irradiating an aluminum foil with 50 fs pulses of a 0.8 um wavelength laser beam[63]. Three main steps contribute in laser ablation synthesis method and formation of nanoparticles from a target immersed in liquid. Laser pulse, first, heats up the target surface to the boiling point, and thus, plasma plume containing vapor atoms of target is generated. Then, plasma expands adiabatically; and finally, nanoparticles will be generated when condensation occurs. Synthesis parameters such as laser wavelength, laser energy, pulse width, liquid media type, and ablation time can notably affect the product characteristics. The average size of Al nanoparticles formed lies between 10 and 60 nm, depending on the experimental conditions.

One problem with this method is that in long ablation times, the ablation rate decreases. It occurs when high concentrations of nanoparticles in produced colloidal solution blocks the laser path, and thus, a part of laser energy is absorbed by formerly synthesized nanoparticles instead of the target surface [64].

3.4.3 Solution reduction
In 2012, Al nanoparticles were synthesized by solution reduction process successfully. They used from benzildiethylenetriamine as a reducing agent in methanol, ethanol, water, acetonitrile, cyclohexane and dimethylsulphoxide. The best results obtained by the ethanol for the synthesis of Al nanoparticles in the range of 4–13 nm. Also Al nanoparticles (5–8 nm size) have been synthesized by using NaBH4 or LiAlH4[65]. In another study, it reported the synthesis of Al/Au bimetallic nanoparticles in water solution. They used from Al+3 and Au+3 metal salts and reducing solution contain sodium citrate, tannic acid, and sodium carbonate [66].

3.4.4 Gas-phase synthesis
Gas evaporation
The most common method to synthesize aluminum nanoparticles is the evaporation of aluminum from the molten state into a chamber filled with an inert gas, where the gaseous metal condenses. The purity of the aluminum starting material, and the type and purity of the inert gas atmosphere, strongly influence the properties of the aluminum nanoparticles obtained [67,68]. A modified inert gas evaporation method called cryomelting can also be used to make aluminum nanoparticles. In the cryomelting process, the evaporated metal is rapidly condensed in regioncooled to about 70 K. This method can produce 20 – 500 nm aluminum nanoparticles in which 60% of the particles are smaller 70 nm in size, as observed by TEM [69].

3.5 Platinum: Stable aqueous colloidal platinum nanoparticles are prepared by citrate reduction. The method was first used by Furlong who prepared platinum nanoparticles as small as 4 nm [54]. It was found that increasing heat during the reaction causes an increase in particle size. Henglein et al. produced different Pt colloidal sols by utilizing radiolysis, hydrogen and citrate reduction [70]. Particles with average diameter of 1.8,2.5, and7.0 nmare obtained by Radiolysis, citrate and hydrogen reduction. The citrate acts not only as a reductant, but also as a stabiliser for Pt colloidal sols [71]. Lin et al. shapedcitrate-stabilised platinum nanoparticles of 2-3 nm average size with approximately +_ 2 nm distributionvia methanol reduction. Luo and Sum devised a single-step heat-treatment

method for the production of poly(vinylalcohol) (PVA) stabilised platinum nanoparticles with diameters of 2-7 nm. The PVA acted as reductant and stabiliser [72, 73].

3.6 Ruthenium: Vladimir and his team synthesized 20 wt.% Ru/C under argon using dry solvents [114]. In a 2-L two neck flask fitted with a dropping funnel and a vacuum adapter, maintained under a steady flow of argon, 2.868 g of anhydrous ruthenium chloride and 1 L of dry THF were introduced and sonicated for 15min to generate a uniform suspension of the salt. 5.6 g of vulcan XC 72 was added to the suspension, and the mixture was stirred vigorously for 2 h at room temperature. The flask was then placed in an oil bath maintained at 50oC. 27.0mL of 1.5MLiBet3H/ THF solution was dripped over 2 h, and the resulting mass was allowed to stir vigorously for 24 h at 50^{o} C.After stopping the stirring the flask was allowed to cool to room temperature. Colorless and clear supernatantwas pressed off and the precipitate was washed twice with 150mL portions of THFand dried. The residue was conditioned at 200^{o}C using argon (5min) followed by hydrogen (30min) and argon (5 min) again. The sample tube was allowed to cool to room temperature. This enabled the formation of stable, 20 wt.% Ru/C catalyst [73].

4. Characterization

An initial characterization of the test substance is imperative before any toxicity screening is commenced. A more extensive and complete characterization, including size distribution, shape, surface area, surface chemistry, crystallinity, porosity, agglomeration state, surface charge, solubility, etc., is recommended for nanomaterials in order to determine the correct correlation between their physicochemical properties and the biological effects they elicit. Proper characterization prior to the experiments ensures more repeatability and hence greater reliability of results [74-77]. In addition, the characteristics of commercially available particles that are specified by the manufacturer sometimes differ from those found by the researcher [78].

4.1 Brunauer-Emett-Teller (BET) method:

The BET method is typically used to calculate the surface areas of solids through the physical adsorption of gas molecules onto the solid surface. It involves adsorbing a liquid nitrogen monolayer onto the surfaces of particles and then measuring the amount of nitrogen released upon vaporizing that layer. Thus, the BET surface represents the surface area that is freely accessible to gases. The primary particle diameter (assumed to be the equivalent sphere diameter) is then calculated from the specific surface area and the density of the particles—data that are already available. [77, 79].

4.2 Atomic force microscope:

An atomic force microscope (AFM) is a cost-effective instrument that has several advantages in the characterization of nanoparticles.

Qualitative Analysis:

Using the AFM, individual particles and groups of particles can be resolved. Microscope images are essential in research and development projects and can be critical when troubleshooting quality control issues. The AFM offers visualization in three dimensions. Resolution in the vertical, or Z, axis is limited by the vibration environment of the instrument: whereas resolution in the horizontal, or X-Y, axis is limited by the diameter of tip utilized for scanning [80].

Quantitative analysis:

Software-based image processing of AFM data can generate quantitative information from individual nanoparticles and between groups of nanoparticles. For individual particles, size information (length, width, and height) and other physical properties (such as morphology and surface texture) can be measured [81].

4.3 Electron microscopy:

Electron microscopy is the simplest and most widely used technique that directly measures particle size, size distribution and morphology. However, it is time consuming and requires a sufficient number of particles containing the fields to be analyzed before a sound statistical assessment can be made [81].

4.3.1 Transmission electron microscopy/ scanning electron microscopy:

TEM/SEM analysis is generally performed in vacuum, whereas the characterization of nanoparticles by AFM can be performed in ambient air and in liquid dispersions, which may be very advantageous for biological studies. AFM scans also offer a wider range,

and particles from 1 nm to 8 μm can be measured in a single scan [82]. Moreover, it requires much less laboratory space than TEM/SEM and is simpler to operate.

4.4 X ray diffraction:

The particle size is calculated on the base of Debye Scherrer equation: $Dp=0.9\lambda/\beta \cos\theta$,

Where Dp corresponds to the particle width in A^o, λ is the Xray wavelength, θ is the Bragg angle, and β corresponds to the full width at half maximum (fwhm, in radians) of the peak under consideration. When the samples are heated to 200^{o}C for 3 h, the peaks become well-defined and sharp due to increased crystallinity. On heat treatment the particle size increases threefold for all bimetallic combinations [83-86].

4.5 Dynamic light scattering (DLS):

DLS measures time dependent fluctuations in scattering intensity produced by particles in Brownian motion, and yields the size of the particle by applying the Stokes–Einstein relation. The size obtained by DLS is usually greater than that measured by other techniques, like TEM, BET, etc. This can be attributed to the fact that DLS measures Brownian motion and the subsequent size distribution of an ensemble of particles in solution and yields the mean hydrodynamic diameter, which is usually larger than the BET or TEM diameter as it includes a few solvent layers [87]. During DLS measurements, there is a tendency of particles to aggregate in the aqueous state, so this method gives the sizes of clustered particles rather than individual particles. DLS reports an intensity weighted average hydrodynamic diameter of a collection of particles, so any sample polydispersity will skew the average diameter towards larger particle sizes [87].

4.6 Nanoparticle Tracking Analysis (NTA):

A more recently developed system based on the Brownian motion of nanoparticles is known as nanoparticle tracking and analysis (NTA). This allows nanoparticles to be visualized individually with simultaneous analysis of their Brownian motion. The particle size distribution can be obtained on a particle-by-particle basis, allowing higher resolution and therefore a better understanding of aggregation than ensemble methods like DLS. It avoids any intensity bias towards large particles that could result in a small number of large particles/agglomerates masking the presence of a greater number of nanoscale particles, as seen with other light-scattering techniques (e.g., DLS). NTA can be used to identify and count nanoparticle aggregates/ agglomerates due to its ability to visualize the particles individ ually [88].

5. Toxicity of nanoparticles:

Nanoparticles require much more extensive particle characterization (of factors such as size, shape, solubility, agglomeration, elemental purity, surface area, etc.) than other chemical compounds.

5.1 Dose-dependent toxicity

Dose is defined as the amount or quantity of substance that will reach a biological system. The dose is directly related to exposure or the concentration of substance in the relevant medium (air, food, water) multiplied by the duration of contact. Generally, the negative health effects of nanoparticles do not correlate with nanoparticle mass dose [89-90]. Comparing the health effects of inhaled TiO2 nanoparticles with different sizes, it is remarkable that the low dose (10 mg/m3) exposure to 20 nm diameter particles resulted in a greater lung tumor incidence than the high dose (250 mg/m3) exposure of 300 nm diameter particles. The measure that correlates with the effects is the surface area and not the mass dose [90,93,94].

5.2 Size-dependent toxicity

In the last decade, toxicological studies have demonstrated that small nanoparticles (<100 nm)cause adverse respiratory health effects, typically causing more inflammation than larger particles made from the same material [89], [90], [91], [93], [94], [94]. In the postexposure period (up to 1 year) it was observed that the smaller particles had (1) a significantly prolonged retention, (2) increased translocation to the pulmonary interstitium and pulmonary persistence of nanoparticles, (3) greater epithelial effects (such as type II cell proliferation), (4) impairment of alveolar macrophages function [92].

5.3. Surface area-dependent toxicity

For the same mass of particles with the same chemical composition and crystalline structure, agreater toxicity was found from nanoparticles

than from their larger counterparts. This led to theconclusion that the inflammatory effect may be dependent on the surface area of nanoparticles,suggesting a need for changes in definitions and regulations related to dose and exposure limits. Indeed, smaller nanoparticles have higher surface area and particle number per unit mass compared to larger particles. The body will react differently to the same mass dose consisting of billions of nanoparticles compared to several microparticles. Larger surface area leads to increased reactivity [98] and is an increased source of reactive oxygen species, as demonstrated by in vitro experiments [90].

5.4. Concentration-dependent toxicity

There are many contradictory results related to the toxic effects of nanoparticles at different concentrations. Some studies show that certain materials are not as toxic as was observed by other studies. When comparing the results of different studies one must take into account that there are differences in the aggregation properties of nanoparticles in air and water, resulting in inherent discrepancies between inhalation studies and instillation or in vitro experiments. The aggregation may depend on surface charge, material type, size, among others. One must stress the fact that aggregation of nanoparticles is essential in determining their toxicity, due to a more effective macrophage clearance for larger particles compared to smaller ones (that seem to evade easier this defense mechanism), leading to reduced toxicity of nanoparticle aggregates larger than 100-200 nm [89], [95]. It has been demonstrated that a high concentration of nanoparticles would promoteparticle aggregation [93], [95], and therefore reduce toxic effects compared to lower concentrations [97]. Most aggregates are observed to be larger than 100 nm, a size that seems to be a threshold for many of the adverse health effects of small particles. Therefore, experiments performed with high concentrations of nanoparticles will lead to the formation of nanoparticle aggregates that may not be as toxic as lower concentrations of the same nanoparticles.

5.5 Toxic effects of various nanoparticles:

The Gold NP's affects cellular micromotility; mitochondrial damage; oxidative stress, autophagy in in vitro studies whereas in In vivo studies there is bioaccumulation in important body organs; acute inflammation and apoptosis in the liver; adverse effect on human sperm motility; penetration of gold nanoparticles into sperm head and tail. [97-101]. Silver NP's there is cytotoxicity and chromosome instability, oxidative stress, apoptosis, intracellular calcium transients, cell cycle arrest, interference with DNA replication fidelity, free radical-induced oxidative stress and alteration of gene expression; blood–brain barrier destruction and astrocyte swelling, neuronal degeneration; induce brain edema formation. There is Cytotoxicity and genotoxicity in fish cells; NPs accumulate in gill tissue; adverse effects on embryonic development of oyster, lysosomal destabilization of adult oysters; oxidative stress, double-strand break marker gamma-H2AX and the expression of p53 protein, embryonic morphological malformations in zebrafish. In Drosophila melanogaster there is induced heat shock stress, oxidative stress, DNA damage and apoptosis, with upregulation of p53 and p38 proteins. Decreased reproduction potential is seen in Caenorhabditis elegans. [102-117].

Even Beryllium toxicity can lead to hypersensitivity, allergic reaction characterized by an inflammatory immune response. Hypersensitivity can lead to chronic beryllium disease, where white blood cells accumulate around absorbed beryllium particles and form granulomas leading to anorexia, weight loss, cyanosis of the extremities, and heart enlargement. Long-term exposure to beryllium causes cancer in animals and increased risk of lung cancer in humans [118, 119].

Exposure to high levels of lead and its compounds can cause serious disability. At highest risk are workers involved in the manufacture of batteries, metals, and paints; the printing industry; or chronically exposed to lead dust (e.g. through sanding of surfaces coated with lead) or insecticides. Inhaled or ingested lead circulates in the blood and is deposited in bone and other tissue Manifestations of lead intoxication include impairment of mental functions, visualmotor performance, memory, and attention span, as well as anemia, fatigue, lack of appetite, abdominal pain, and kidney disease, among others. [120]. Exposure to Cobalt can also cause asthma, acute illness (fever, anorexia, malaise, and difficulty breathing, resembling a viral illness), and interstitial pneumonitis [118,

121]. High- dose exposure of Cadmium can lead to severe lung irritation, nausea and vomiting. Long-term low-dosage exposure in humans causes lung emphysema, impairment of the immune system, central nervous system and liver damage. Occupational exposure to cadmium has been linked to lung cancer in humans [120, 122].Exposure to Aluminum occurs through consumption of food and water, as well as usage of many products containing aluminum, including antacids and anti-perspirants. The use of antiperspirants combined with under-arm shaving is associated with an earlier age of breast cancer diagnosis. Excess of Aluminum can lead to anemia, bone disease, and dementia. Exposure to high levels of aluminum (and other metals, such as iron) is related to neurological disorders, such as dialysis encephalopathy, Parkinson dementia, and especially Alzheimer's disease [123-125].
Occupational exposure to nickel via inhalation of dust and fumes is associated with cancers of lung and sinus. Chromium derived from smelting has also been found to cause cancer [120]. Manganese is both an essential nutrient and is known to have neurotoxic effects.At high levels, manganese exposure to contaminated water or through inhalation results in neurological impairment. Occupational exposure generally occurs only to those involved in mining and welding. The neurological disorder linked most closely to manganese is Parkinson's disease [126,127].

In studies of animals administered excessive amounts of iron, orally and by injection, showed an increased risk of of adenocarcinomas, colorectal tumors, hepatomas, mammary tumors, mesothelioma, renal tubular cell carcinomas, and sarcomas. In humans, injection of iron compounds has been shown to cause sarcomas at the sites of deposition. Patients with hemochromatosis (genetic disease characterized by increased iron absorption) have an enhanced susceptibility to liver cancer. The accumulation of iron in brain regions with decreased function, and cell loss has been observed in many neurological diseases, such as Parkinson's disease, Alzheimer's disease, etc. Inhalation of iron dust causes a respiratory disease called pneumoconiosis [121, 127, 128].

6. Regulation of metal nanoparticles

6.1 Why there is a need of regulation?

- Global R & D funding in Nano S & T is increasing and reached US $20 billion/yr
- Nanotechnology found to have potential applications in almost all spheres of human activity including household, medical, industrial and military
- Nanotechnology based consumer products are growing year after year (54 in 2005 to 1317 in 2010)
- The market for nanotechnology products & services are expected to reach 1.5 trillion by 2015
- Diversity of materials and applications, surrounded with uncertainty and lack of adequate information about its impacts on safety, health and environment
- Lack of standardization in nomenclature, metrics, and test materials for assessment of nanomaterials [129].

6.2 Regulation in India [130,131,132]

In India, the government has been playing a central role in promoting nanotechnology research and development, and application. The role of the state is also of prime importance in defining regulatory objectives. The current focus of government action on promotion is evident from the mission mode it has approached under Nano Mission. Although there does not exist a nanotechnology specific regulation currently, there exists a whole range of regulatory instruments that do and will extend to the nanotechnology applications in India.

A regulatory matrix comprising several central Acts, rules and notifications pertaining to intellectual property rights and environment, health and safety for nanotechnology development and applications in India has been developed and categorized under the following broad heads -

- Research and development
- Production and marketing
- Occupational health and safety
- Environmental health
- Waste disposal

Institutionally, Ministry of health and family welfare (MoHFW) is in charge of prevention and control of health related hazards although it has been included under Indian Council of Medical Research (ICMR). Another level, at which the ministry plays an instrumental role, is

that of regulating drugs and pharmaceuticals through the Central Drugs Standards Control Organization. The main challenges faced by regulatory institutions currently, or the ones which are likely to make the task of regulating nanotechnology difficult, relate to the following:

- **Regulatory capacity**
- **Information asymmetry**
- **Interagency coordination**
- **Overlapping roles and mandates**

Another challenge for the regulatory institutions is to keep pace with international developments, in terms of responding, incorporating and even influencing them.

One of the outcomes of the focus on technology development as part of the state development agenda has been the setting up of individual departments at the level of central government with a view of promotion of specific technologies, thus we have the Department of Biotechnology, Department of Atomic Energy and the Department of Information Technology. The setting up individual departments is to provide strategic leadership and guidance for technology development in the specific sectors like ICT and Biotechnology as well as the primary objective of the department is to promote and facilitate the development of that technology. The Ministry of Science and Technology administers its functions through three departments – department of science and technology (DST), department of biotechnology (DBT) and department of scientific and industrial research (DSIR). Of these DST is the most important one with the objective of objective of promoting new areas of science and technology and to play the role of a nodal department for organizing, coordinating and promoting S&T activities in India. Currently, DST is the most instrumental wing of government for providing a thrust to nanotechnology development. The Department, engaged with the agenda of promoting nanotech as a thrust area, has declared large investments for basic and applied research promotion, infrastructure support, education and international collaboration under the Nano Mission started in 2007.Speaking at the 93rd Indian Science Congress, Prof CNR Rao, chairman of the Nano Mission Councel said, “If we don't join the (nano) race, we will be left behind”. It is this fear of being left behind by other countries in the nano revolution that has triggered a single point agenda for giving a thrust to nanotechnology R&D and application. [130].

Nano Mission is an umbrella programme implemented by DST for capacity building towards overall development of the field of nanotechnology research in India. Of the total proposed outlay of Rs. 19,300 crores for Department of Science and Technology under the XI five-year plan, Rs. 1000 crores have been assigned for the nano mission. There are certain public private initiatives in the form of industry-linked projects under the Mission, half of which are with companies dealing with drugs and pharmaceuticals [130, 131].

Defence Research and Development Organization (DRDO) which has fullerenes & Nano tubes as one of the thrust areas in Materials research, has developed diagnostics tools for TB and typhoid, by using nanotechnology [130,132]

In order that nanotechnology and nanomaterials can be developed responsibly, with optimization of benefits and minimization of risks, international cooperation on identifying and resolving gaps in knowledge is required. It is recognized that a major barrier to progress in this area is the confidential nature of much of the research on nanoparticles. Means of facilitating co-operation with industry to fill some of the critical knowledge gaps for risk assessment purposes need to be found to avoid the experience of the biotechnology industry of public perception of the risks. [133]. A transparent framework for risk benefit analysis should also be developed that is able to achieve wide acceptability [133].

7. Current trends of metal nanoparticles

Metal oxide nanoparticles offer a variety of functionalities that remain desirable over a number of segments such as anti-corrosion, anti-bacterialism, thermal barrier, easy-clean, UV- absorbent & combinations thereof. Increasing research & development activities to enhance the product applications coupled with the rapid growth in the nanotechnology is anticipated to drive the metal and metal oxide nanoparticles market, and furthermore support its application in pharmaceuticals, medical and cosmetics over the forecast period. Owing to

novel optical, electronic, chemical catalytic, magnetic and mechanical properties from high surface to volume ratio, and quantum size effects, these metal and metal oxide nanoparticles are being applied across a raft of high-tech technologies and industries. The global metal and metal oxide nanoparticles are used in Consumer electronics, Paints and coatings, Automobiles, Construction, Aerospace, Medicine, Packaging, Others (Sporting goods, household cleaning, food & beverages, agriculture) [134]. Furthermore, construction, automotive, packaging and aerospace industries are expected to witness significant growth on account of strong support from the developing countries including China, Brazil and India. North America is currently the largest market in metal and metal oxide nanoparticles, owing to the high venture capital investments in research & development activities, to enhance the metal and metal oxide nanoparticles applications. Some of the key participants identified in the global metal and metal oxide nanoparticles are Altair Nanomaterials, Inc., Sigma-Aldrich Co. LLC., American Elements, Access Business Group, US Research Nanomaterials Inc., Reinste Nano Ventures, NanoScale Corporation, and EPRUI Nanoparticles & Microspheres Co. Ltd., among many others [134]. Global market for Platinum Nanoparticles is likely to witness growth on account of increasing expenditure in automotive, chemical and healthcare industries for development of nanotechnology. Growing requirement of metal nanoparticles such as gold, silver and platinum for catalysis is expected to fuel the nanotechnology industry growth. Increasing demand for nanoparticles in medical industry for diagnostics and therapeutics is expected to have a positive impact on the platinum nanoparticles market. Development of metal nanomaterials for drug delivery system for cancer treatment coupled with investments in R&D for fillings for nanowires is expected to have a promising affect on metal nanomaterials industry. Advanced research and development on metal nanoparticles with combination of other metals including nickel and tungsten is expected to have significant results on solar technology.In 2015, Government of China published a patent on application of platinum metal nanomaterials and tungsten trioxide for development of dye sensitized solar cells technology [135]**.** Key players include BBI Solutions, Tanaka, Sigma Aldrich, Nanosphere, Solaris Nanosciences, NanoBio Chemicals Pvt Ltd and Nanopartz Inc. [135].

Aluminum Nanoparticles are being used on a large scale as compared to bulk materials due to its enhanced properties. The various application areas of aluminum oxide include pharmaceutical and industrial manufacturing processes among others. It is used as an adsorbent, catalyst, desiccating agent, used in manufacture of dental cement, as a dispersing agent in food, as an abrasive in toothpaste and also has uses in hemodialysis. Other applications include fillers for plastics, purifier to remove water from gas streams and for reflective decorative effects and paints. In addition it is used as a medium of chromatography in sodium vapor lamps, to make spark plug insulators, as a material in hip replacements and also used in compact fluorescent lamps. Aluminum oxide has high demand as a biomaterial for medical transplants in the market. Growing pharmaceutical industry along with rising demand for aluminum oxide from other applications in the industry is expected to be the major driver for aluminum oxide. The growing demand from ceramic industries to manufacture porous ceramics is also expected to boost demand for aluminum oxide in the near future. However, exposure to aluminum oxide causing health concerns is expected to be the major restraining factor for the growth of aluminum oxide market. Aluminum oxide is not a human carcinogen but is noted to develop severe pulmonary reactions such as fibrosis, pneumothorax and emphysema. Short-term exposure to aluminum oxide may cause upper respiratory tract and eye irritation. Long-term exposure may affect the central nervous system. [138]. Key palyers are Almatis, Chemicals India Company, Khambhalay Abrasive, Tirupati Industries, Snam Abrasives Pvt. Ltd, MTC Wesgo, Nivaka Pharmaceuticals Industries, SASOL, GRACE, GIFA, Russel and Vega among others [138].

The global Gold Nanoparticles market is expected to witness significant rise owing to advancements in the field of nanotechnology coupled with growing requirements of metal nanoparticles in numerous end-use industries. Increasing demand for nanotechnology based

diagnostics and therapeutics in medical industry is anticipated to fuel gold nanoparticles market growth over the forecast period. Technological advancements in the field of nanotechnology for medical diagnostics are also anticipated to impact the quality and performance of finished products. Growing demand for nanotech products especially for cancer and tumor treatment & diagnosis has led to the development of metal nanoparticles for drug delivery systems. Global nanotechnology market has witnessed an increase in intellectual property filings concerning cancer targeting nanoparticles technology and nanowires. Increasing investments in R&D coupled with above mentioned trends is expected to have a positive impact on gold nanoparticles market over the next few years. Gold nanoparticles find applications in several other industries such as electronics and chemical catalysis. Surging demand for high efficiency compact mass storage media devices such as CDs, DVDs, USB drives and flash cards has led to the increase in application of metal nanoparticles. Enhanced stability and lower crystal growth of molecules provides gold nanoparticles an edge over other metal nanoparticles such as silver and platinum. [137]. Increasing R&D spending for cancer targeting nanoparticle technology and drug delivery systems is estimated to have a positive impact on gold nanoparticles market growth over the next six years. Global demand for nanotechnology medical products grew at a CAGR of 17% from 2009 to 2014 and the segment is anticipated to show similar growth over the next six years. This high growth of nanotechnology medical products along with increasing application scope of gold nanoparticles in diagnostic tests for a wide range of diseases including cancer, HIV/AIDS, and cardiovascular diseases is estimated to drive global demand in the medical industry over the next six years. Electronics segment is estimated to have the highest growth rates over the forecast period on account of growing demand for compact storage devices and increasing nanotechnology usage in photovoltaic. Growth of niche applications such as fruit & vegetable protection and nanowires for biosensors is expected to create immense future opportunities for gold nanoparticles market growth. Global gold nanoparticles market is characterized by high competition among the market players and low yield coupled with high investments in R&D. Market participants compete on the basis of product development for specific end-use applications. Major companies operating in global gold nanoparticles market include BBI Solutions, Cytodiagnostics, Goldsol, NanoHybrids Corp., Nanopartz Inc., Nanosphere, Nanostellar, Tanaka, Metalor, Solaris Nanosciences, NanoRods LLC, Innova Biosciences, Sigma Aldrich, Appolo Biolife and NanoBio Chemicals India Pvt. Ltd [137].

Conclusion

Nanoparticles owing to their smaller particle size and large surface area can be easily manipulated to achieve both passive and parenteral administration. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects. However the smaller particles have (1) a significantly prolonged retention, (2) increased translocation to the pulmonary interstitium, (3) greater epithelial effects (such as type II cell proliferation), (4) impairment of alveolar macrophages function. As they have higher surface area and particle number per unit mass compared to larger particles the body react differently to the same mass dose consisting of billions of nanoparticles compared to several microparticles. Larger surface area leads to increased reactivity and is an increased source of reactive oxygen species are produced leading to toxicity. Metal and metal oxides of nanoparticles are widely used in our health sector nowadays. They are so widely used now that we require regulatory guidelines for them. In India, regulation of metal nanoparticle is not that established but our Ministry of health and family welfare is trying to establish certain guidelines for the regulatory purposes and have appointed certain Departments for same. Even Nano Mission has been started by our government. Nano Mission is an umbrella programme implemented by DST (Department of Science Technology) for overall development of the field of nanotechnology research in India.

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