

Synthesis, Characterization and Biomedical Applications of Nanoparticles

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ABSTRACT

Nanoscience and nanotechnology have found their way into the fields of biotechnology and medicine. The nanomaterials level is the most advanced at present, both in scientific knowledge and in commercial applications. Nanoparticles by themselves offer specific physicochemical properties that they do not exhibit in bulk form, where materials show constant physical properties regardless of size. These materials are superior and indispensable in many areas of human activity due to their unique size-dependent properties. An approach was made herein to review the synthetic strategies of nanoparticles, their characterization techniques and applications in the field of biology.

Key words: Nanoparticles, physicochemical, synthetic strategies, characterization, applications, biology

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INTRODUCTION

In recent years, tremendous research activities are targeting at the development and advancement of a new interdisciplinary scientific field-nanoscience; the science of the extremely tiny particles. Nanotechnology development is the need to understand the techniques for atomic and molecular based study of matter in nanoscale¹. Nano, the Greek word for dwarf, is the prefix for units of 10⁻⁹ and the measurement at this level is in nanometer (nm). Nanotechnology takes benefit of the fact that when a solid material becomes very minute, its specific surface area increases, which leads to an increase in the surface reactivity and quantum-related effects. These properties make nanoparticles applicable in many biomedical applications. The excellent properties of these materials offer a very promising future for their use in the field of life sciences^{2,3}. Thus, the unusual properties have attracted enormous attention of researchers from nearly every field of science including biology and medicine. Exploration of novel properties of nanoparticles and their application has become very active area of research. Nanotechnology is at the leading edge of the rapidly developing new therapeutic and diagnostic concepts in all areas of medicine. Nanoparticles have now entered a commercial exploration period⁴. There is an increasing brightness that nanotechnology will bring considerable advances in the diagnosis and treatment of disease as applied to medicine. The exclusive properties and

efficacy of nanoparticles arise from a variety of attributes. It includes the similar size of nanoparticles and biomolecules such as proteins and polynucleic acids. So, these nanoparticles can offer unique interactions with biomolecules both on the surface of and inside the cells, which may develop cancer diagnosis and treatment.

The aim of this review is firstly to give reader a preview of some of the synthesis methods (reverse micelle process⁵, salt reduction⁶, microwave irradiation⁷, solvothermal analysis⁸, electrochemical synthesis⁹, arc discharge method, pulsed wire discharge method, thermal decomposition method and biological synthesis) of nanomaterial, secondly, the characterization of nanoparticles using Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), X-Ray Diffraction (XRD), X-ray Absorption Spectroscopy (XAS), Small Angle Neutron Scattering (SANS) and Fourier IR spectroscopy (FTIR). In this article, we will also explore some of the biomedical applications such as targeted drug delivery, hyperthermia cancer treatment, tissue engineering and separation and purification of biological molecules and cells.

METHODS OF SYNTHESIS

A variety of chemical methods have been employed for the synthesis of inorganic nanoparticles. The techniques for producing nanoparticles fall into three main categories: solid, vapour and solution¹⁰. Particles can then be coated with hydrophilic or hydrophobic substances in accordance to the desired application.

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The solid state processes method involves the mechanical milling or grinding of raw powder to create nanoparticles. The properties of the resultant nanoparticles are affected by the milling material, milling time and atmospheric medium. This route can be used to fabricate nanoparticles from materials that don't eagerly lend themselves to the other two techniques. Contamination to the products from the milling equipment as well as producing particles with wide size distribution can be a problem. The Vapour phase fabrication approach is used to make metallic and metal oxide ceramic nanoparticles. It involves the formation of a supersaturated vapor of condensable gaseous species as a result of a chemical reaction. This is followed by rapid condensation that will reduce the vapor pressure of the condensable species to create nanosized clusters. Various approaches can be employed to vaporize the metal and the nature and size of the nanoparticles depends on the medium into which the vapour is released. Figure 1 shows the schematic representation of preparation of nanoparticles and nanoclusters by vapour phase technique.

In the liquid phase fabrication technique, the chemical precipitation usually occurs from homogeneous solution. There occurs nucleation when the concentration of the ingredient precursor reaches critical supersaturation followed by a period of uniform growth as the solutes diffuse from the solution to the surface of the nuclei until the final size is reached. This process requires the partition of the nucleation and growth phases, with no more nucleation occurring during the growth period to attain monodisperse nanoparticles. Multiple nucleation proceedings can result in tiny particles aggregating to form much larger particles and typically leads to the coarsening of the size allocation of the particles¹¹. This process is known as Ostwald ripening. Chemical precipitation can be made more favourable method for producing uniform particles by better controlling nucleation, particle growth and

particle interaction in a liquid medium. The schematic representation for the fabrication of nanoparticles by liquid phase technique is shown in the Fig. 2.

Chemical reduction method: Reduction is the transfer of electrons from reducing agent to oxidized metal species. It is the most often used method for the chemical synthesis of nanoparticles which deals with the reduction of metal particles to nanoparticles using chemical reducing agents such as sodium borohydride or sodium citrate. The reducing agent can form an intermediate with the oxidized metal species without altering its oxidation state. In this situation, the reduction process can be initiated by rising the temperature, for example and it can be conducted very gradually, producing conditions that lead to the creation of highly crystalline structures of regular shape. An example of this is the formation of silver nanoparticles by chemical reduction method in which silver nitrate is taken as the metal precursor and hydrazine hydrate as a reducing agent¹².

Microwave irradiation: Microwave irradiation is one of the novel techniques developed recently for the synthesis of nanoparticles. Microwaves are a form of electromagnetic radiations, with frequencies in the range of 300 MHz to 300 GHz. The commonly used frequency in this route is 2.456 GHz. In the microwave method of synthesis, microwave radiations are introduced in the reaction solution. An example is the microwave-assisted synthesis of copper nanoparticles that has become popular due to its simplicity, ease of operation, rapid volumetric heating and kinetics, short reaction period and increasing yield of products compared to the conventional heating methods¹³.

Solvothermal synthesis: In the solvothermal processes, the chemical reaction takes place in a sealed vessel such as bomb or autoclave, where solvents are brought to

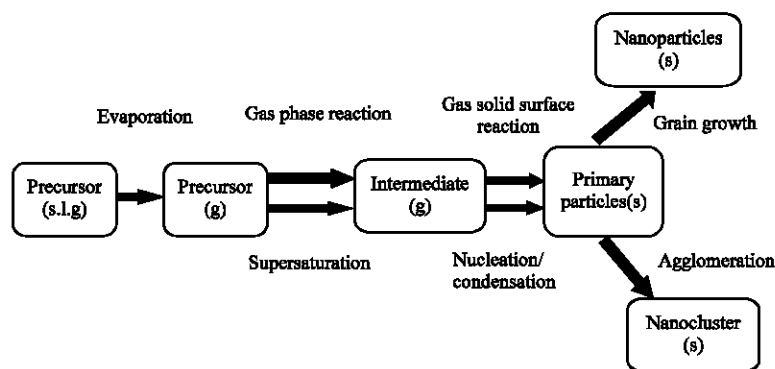


Fig. 1: Schematic representation of preparation of nanoparticles and nanoclusters by vapour phase technique

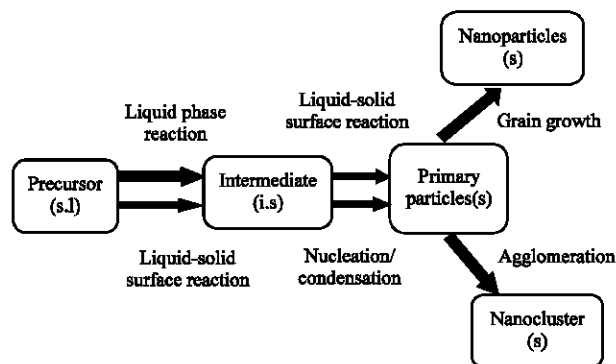


Fig. 2: Schematic representation for the fabrication of nanoparticles by liquid phase technique

temperatures well above their boiling points. When water is used as solvent, it is called a hydrothermal process. Singh *et al.*¹⁴ used solvothermal reduction process for the preparation of nanoparticles. The reaction was initiated under ambient conditions before the mixture was transferred to a teflon lined sealed stainless steel autoclaves and heated to 50°C for 6 h under autogenous pressure. The effect of reaction temperature, concentration of precursor and time of growth plays vital role on the properties of fabricated nanoparticles.

Electrochemical process: In this method, chemical reaction is induced in an electrolyte solution with the use of an applied voltage. Electrochemical synthesis is carried out by passing an electric current between two electrodes which are separated by an electrolyte. The synthesis of nanoparticles takes place at the electrode-electrolyte interface. This technique provides low costs, simple operation, high flexibility, easy availability of equipment instruments, less contamination (pure product) and environment-friendly (eco-friendly) process. A wide variety of nanomaterials could be synthesized using this method. Much research work has been done on the electrochemical technique in advancing the fundamental understanding and industrial applications, but still many aspects of this technique are under study.

Arc discharge method: This is a physical method for the synthesis of nanoparticles. Two graphite electrodes acting as cathode and anode are used in this method. These electrodes are dipped in metal salt solution. An arc is struck by bringing the electrodes in contact. The synthesis of nanoparticles is carried out at an open circuit and an optimized current.

Pulsed wire discharge method: Pulsed wire discharge is a physical technique to prepare nanoparticles¹⁵. In this method, a metal (copper) wire is evaporated by a pulsed current to produce a vapor, which is then cooled by an

ambient gas to form nanoparticles. This method has advantage of high fabrication rate and high energy efficiency. Moreover, a simple apparatus consisting of a vacuum chamber, a powder collection filter and a discharging circuit is used to prepare nanoparticles. This process is mostly useful for those metals of high electrical conductivity that are easily available in the thin wire form¹⁶. This technique was used by Sen for the preparation of metallic nanoparticles such as copper, silver, iron and aluminum nanoparticles with resulting nanoparticles in the range of 20-100 nm while the copper nanoparticles prepared by this technique were of 27-72 nm in size.

Thermal decomposition method: This method is used for the fabrication of monodisperse nanoparticles. An example is the synthesis of ZnO nanoparticles with crystallite sizes of 30-40 nm by a direct thermal decomposition of zinc acetate in air at 400-700°C for 4 h. The method is fast, simple and cost effective and provides a promising synthesis route for preparations of metal oxide and complex oxide nanoparticles.

Biological synthesis: As the physical and chemical processes are costly and hazardous there arises a need for biosynthesis of nanoparticles. Therefore, scientists used microorganisms and then plant extracts for the synthesis in the search for cheaper routes for nanoparticle synthesis. Biological agents used for the synthesis of nanoparticles consist of primarily microbes and plants¹⁷. Metal compounds usually reduce into their respective nanoparticles because of microbial enzymes or the plant phytochemicals with antioxidant or reducing properties. The biological methods used for the preparation of nanoparticles include both intracellular and extracellular methods¹⁸. The intracellular synthesis method usually involves the use of bacteria and actinomycetes. The bacterial cell filtrate is treated with metal salt solution and kept in a shaker in dark at ambient temperature and

pressure conditions¹⁹. For the extracellular synthesis of nanoparticles using bacteria, the bacterial culture is centrifuged at 8000x g and the supernatant is challenged with the metal salt solution. The biosynthesis of nanoparticles involves simple preparation protocols and a smaller amount of toxicity and includes a broad scope of applications according to their morphology. Nanosized copper particles are biosynthesised by protein-mediated process²⁰. In this method, the protein solution with copper sulphate solution was reduced with sodium borohydrate. Argon gas was used as inert medium during the process to avoid oxidation.

Characterization techniques: It is very important to characterize the nanoparticles to understand the control of synthesis and their applications. There are various techniques accessible for the characterization of nanoparticles. Microscopy techniques have been commonly employed for particle size and characterization. Some of these techniques are Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Atomic Absorption Spectrophotometry (AAS) is used to check the concentration of metallic nanoparticles. Some of the most common techniques for this purpose are as follows:

Scanning electron microscopy: A Scanning Electron Microscope (SEM) uses a beam of electrons targeted at a specimen. The instrument has features such as an electron gun, condenser lenses and a vacuum system. SEM is principally used to study the surface or near surface structure of bulk specimens. The principle images produced in SEM are of three types: secondary electron images, backscattered electron images and elemental X-ray maps. It is easy to interpret SEM images although the spatial resolution of SEM is approximately 15 nm, which is not as good as TEM, which can have spatial resolutions in the subnanometer range. It is also possible to image a comparatively large area of the specimen with the aid of SEM. The ability to image bulk materials and the variety of analytical modes accessible for measuring the composition and nature of the specimen makes SEM a valuable technique.

Transmission electron microscopy: Transmission Electron Microscopy (TEM) is one of the most common techniques employed to visualize and determines the morphology of the particles. In this technique, monochromatic beam of electrons penetrate a thin specimen, some of which are transmitted through the objective lens and then projected onto a viewing screen (for example, a layer of electron fluorescent material) to generate an image. Particles as small as a few angstroms (10^{-10} m), which is near atomic levels can be viewed in

TEM. High-Resolution Transmission Electron Microscopy (HRTEM) is an imaging mode of the Transmission Electron Microscope (TEM) that allows the imaging of the crystallographic structure of a sample at an atomic scale. Because of its high resolution, it is a valuable tool to study nanoscale properties of crystalline material. At present, the highest resolution realised is 0.47 ångströms (0.047 nm) with double aberration-corrected JEOL R005, Cold Field Emission Gun TEM, at Tokyo Institute of Technology^{21,22}. The HRTEM allows us to obtain information on the crystal planes of the particles and measure the lattice distance.

X-ray diffraction: One of the most conventional techniques that can be used to determine the structure, phases, and average size of the particles is powder X-Ray Diffraction (XRD). In this technique, the structure and the lattice parameters are determined by measuring angle of diffraction when X-rays are made to incident on the powdered specimen. The size of the particles can also be determined from the width of the X-ray peaks using the Scherrer formula²³. The size of the particles estimated from the XRD is seldom found to be more than that from TEM studies because of the broadening of X-ray diffraction lines.

X-ray Absorption Spectroscopy: X-ray Absorption Spectroscopy (XAS) is a widely-used technique for determining the local geometric and/or electronic structure of matter. The experiment is usually performed at synchrotron radiation sources, which provide intense and tunable X-ray beams. XAS data are obtained by tuning the photon energy using a crystalline monochromator to a range where core electrons can be excited (0.1-100 keV photon energy). There are three main regions found on a spectrum generated by XAS data. The dominant feature is called the "rising edge" and is sometimes referred to as XANES (X-ray Absorption Near-Edge Structure) or NEXAFS (Near-edge X-ray Absorption Fine Structure). The pre-edge region is at energies lower than the rising edge. The EXAFS (Extended X-ray Absorption Fine Structure) region is the oscillatory structure above the main edge and corresponds to the scattering of the ejected photoelectron off neighbouring atoms. The combination of XANES and EXAFS is referred to as XAFS. The XANES provides information about the electronic state of the X-ray absorbing atom and the local structure around it. One can get information relating to bond angles, bond lengths, and the presence of adsorbates from NEXAFS. Finally, the EXAFS can give information on the atomic number, distance and coordination number of the atoms surrounding the element.

Small angle neutron scattering: Small-Angle Neutron Scattering (SANS) is an experimental technique that uses elastic neutron scattering at small scattering angles to investigate the structure of various substances at a mesoscopic scale of about 1-1000 nm. During a SANS experiment a beam of neutrons is directed at a sample, which can be an aqueous solution, a solid, a powder, or a crystal. The neutrons are elastically scattered by nuclear interaction with the nuclei or interaction with magnetic momentum of unpaired electrons. Small Angle Neutron Scattering (SANS) is a useful tool to study magnetic nanoparticles. In X-ray scattering, photons interact with electrical cloud but in neutron scattering, neutron interacts with nuclei. The parameters which can be evaluated from SANS data includes the radius of gyration, the particle surface area, shape of the scattering particles, magnetic structure, magnetic correlation, alignment of nanoparticles as well as their response to an external magnetic field²⁴.

Fourier Transform IR Spectroscopy: Fourier Transform Infrared Spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. Transmission spectra for the nanoparticles were obtained by forming thin, transparent KBr pellets containing the materials of interest. The KBr mixtures were placed on a vacuum line overnight before pellet formation and then the pellets were again placed on a vacuum line before use. The transmission spectra were obtained, after purging in dry air, and were background corrected using a reference "blank" KBr pellet. Spectra were obtained for 200 scans at a resolution of 2 cm^{-1} .

Applications: Nanomaterials can be applicable to biology for various purposes.

Drug delivery: Drug targeting has emerged as one of the modern technologies for drug delivery²⁵. This is achieved by attaching a therapeutic drug to a biocompatible magnetic nanoparticle carrier that is injected into the patient via the circulatory system. In the blood stream, by applying a high-gradient, external magnetic field, the drug-carrier complex can be concentrated at a specific site. The medication is then released via enzymatic activity or changes in the physiological conditions²⁶. The possibilities for the application of iron oxide magnetic nanoparticles in drug targeting have drastically increased in recent years²⁷. The magnetic drug carriers have the potential to carry a large dosage of drug to attain high local concentration and avoid toxicity and other adverse side effects arising from high drug doses in other parts of

the organism. A good example is the first biologically interactive agent, albumin-bound paclitaxel, an anti-cancer drug. ABI 007 is encapsulated in a 130 nm NP, designed to avoid solvent-related toxicities and to transport paclitaxel to tumors via molecular pathways involving an endothelial cell-surface albumin receptor and an albumin-binding protein expressed by tumor cells. The paclitaxel is then secreted into the tumor interstitium²⁸. It has been found that the efficiency of many conventional pharmaceutical therapies can be considerably enhanced through drug delivery systems. Generally, therapeutic molecules such as proteins and lipids are encapsulated inside or conjugated to particular carriers composed mainly of lipids and polymers. A high therapeutic efficiency can be achieved without side effects by the encapsulation or conjugation which allows controlled release rather than a burst of drugs.

Hyperthermia: An interesting application of magnetic nanoparticles is in hyperthermia treatment which is considered as a complementary treatment to chemotherapy, radiotherapy and surgery in cancer therapy^{29,30,31}. In hyperthermia treatment of cancers, a device is used to heat malignant cells. But the majority of devices cause coincidental damage to surrounding healthy tissue and hence are restricted in their use. Therefore a possible solution to this problem is use of magnetic nanoparticle hyperthermia. This makes use of dispersion of magnetic nanoparticles into the target tissue and an AC magnetic field of sufficient strength and frequency is applied which results in the heating of the particles. This is followed by transferring of heat produced to the surrounding diseased tissue and if the temperature can be maintained above the therapeutic threshold of 42°C for a time period of 30 min, the tumor is damaged³². The structural properties of the particles (e.g., size, shape) affects amount of heat generated by them. For hyperthermia treatment in cell irradiation, magnetite cationic liposomal nanoparticles and dextran-coated magnetite have been shown to efficiently increase the temperature of tumor cells. This has been anticipated to be one of the key approaches to successful cancer therapy in the future³³. Magnetic hyperthermia has an advantage of allowing the heating to be restricted to the tumor area.

Tissue engineering: Nanoparticles represent a promising tool for therapeutic approaches in bone. Natural bone surface is quite often contains features that are about 100 nm across. The body would try to reject artificial bone plant if its surface were left smooth. This smooth surface produces fibrous tissue covering on the surface of the implant, which reduces the bone-implant contact and as a consequence inflammation may result

due to loosening of the implant. The probability of negative response could be reduced by creating nano-sized features on the bone surface. It also stimulates the production of osteoblasts, the cells which are accountable for the augmentation of the bone matrix and are found on the advancing surface of the developing bone. The effect was demonstrated with polymeric, ceramic and, more recently, metal materials. Titanium is a familiar bone repairing material commonly employed in orthopaedics and dentistry. Its properties are: high fracture resistance, ductility and weight to strength ratio but it do not support cell adhesion and growth well. An Apatite coating on titanium makes it suitable for bonding to bone but the coatings lacks thickness uniformity, strong adhesion and high mechanical strength. Moreover, In order to support the nutrients transport through the cell growth, a stable porous structure is requisite. It was demonstrated that a strongly adherent and uniform non porous layer built of 60 nm crystallites, possessing bioactivity can be obtained by using a biomimetic approach which refers to the slow growth of nano structured apatite film from the simulated body³⁴.

A real bone is a nanocomposite material, composed of hydroxyapatite crystallites in the organic matrix, which is mainly composed of collagen. The bone can recover from mechanical damage as it is mechanically tough. For the purpose of bone tissue regeneration, several non degradable particles, such as silica, lipid, dendrimer, hydroxyapatite, or gold nanoparticles have been used as an effective protein^{35,36,37}. A viscoelastic behaviour (healing) of the human teeth was demonstrated using tribology approach. An investigated hybrid material has been found to improve scratch resistance as well as it possessed a healing behaviour similar to that of the tooth when deposited as a coating on the tooth surface.

Separation and purification of biological molecules and cells: Isolation of biological materials leads to advancements in medical diagnostics³⁸. Proper separation of biological materials finds uses in medical diagnostics and therapeutics, food processing and other industrial purposes. Isolation of biological materials from a biological sample generally involves tedious steps starting from separating the cell to lysis through mechanical action and/or chemical action followed by purification of biological molecules. Conventional isolation of cells and biological molecules involves methods such as magnetic microbeads capture followed by lysis and cesium chloride density gradient centrifugation (which is lengthy and costly) or extraction with phenol (that is harmful). Ethanol precipitation is used to concentrate the nucleic acids for nucleic acid separation which result in lower yields.

It has been established that the efficient quick capture of targeted biological material can be achieved by magnetic Iron Oxide (IO) nanocrystals. The outermost layer of these IO nanocrystals are altered with streptavidin, carboxyl groups, or amines for simple conjugation to biological probes and lessen non-specific binding of unnecessary materials. Presently, scientists are working on a new generation of magnetic separation method to enrich bacteria, stem cells, and tumour cells. The optimization of physical and chemical properties of IO nanocrystals for bulk production is in process. Different types of surface coating strategies are being developed to attain the best coating strategy to achieve a high enrichment factor and high recovery rate of target biological material.

CONCLUSION

An attempt has been made to present a review of the synthesis, characterization, and biological application of nanoparticles. Over the past decade, synthesis of nanoparticles covering a broad range of compositions and tunable sizes has made substantial progress. Nanoparticles present a highly attractive platform for research purpose as well as for a diverse array of biological applications. The surface and bulk properties of these systems makes them applicable for drug delivery, tumour destruction via heating (hyperthermia), tissue engineering and separation and purification of biological molecules and cells. Future research efforts need to be directed towards the development of new synthesis methods of nanoparticles that are designed with a specific biological application.

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