

Synthesis of Nanomaterials

1. Introduction

There are a large number of techniques available to synthesize different types of nanomaterials in the form of colloids, clusters, powders, tubes, rods, wires, thin films etc. There are various physical, chemical, biological and hybrid techniques available to synthesize nanomaterials. The technique to be used depends upon the material of interest, type of nanostructure viz., zero dimensional, one dimensional, or two dimensional material size, quantity etc.

- **Physical methods:** (a) *mechanical*: ball milling, melt mixing
(b) *Vapor*: physical vapor deposition, laser ablation, sputter deposition, electric arc deposition, ion implantation
- **Chemical methods:** colloids, sol-gel, L-B films, inverse micelles.
- **Biological methods:** biomembranes, DNA, enzymes, microorganisms.

2. Physical methods

(a) Ball milling: It is used in making of nanoparticles of some metals and alloys in the form of powder. Usually the mill contains one or more containers are used at a time to make fine particles. Size of container depends upon the quantity of interest. Hardened steel or tungsten carbide balls are put in containers along with powder or flakes (<50 μm) of a material of interest. Initial material can be of arbitrary size and shape. Container is closed with tight lids. The containers are rotated at high speed (a few hundreds of rpm) around their own axis. Additionally they may rotate around some central axis and are therefore called as 'planetary ball mill'. When the containers are rotating around the central axis, the material is forced to the walls and is pressed against the walls. But due to the motion of the containers around their own axis, the material is forced to other region of the container. By controlling the speed of rotation of the central axis and container as well as duration of milling, it is possible to ground the material to fine powder whose size can be quite uniform. Some of the materials like Co, Cr, W, Ni-Ti, Al-Fe, Ag-Fe etc. are made nanocrystalline using ball mill.

Large balls, used for milling, produce smaller grain size and larger defects in the particles. The process may add some impurities from balls. The container may be filled with air or inert gas. However, this can be an additional source of impurity. A temperature rise in the range of 100 to 1100 C is expected to take place during the collisions. Cryo-cooling is used to dissipate the generated heat.

(b) Melt Mixing: It is possible to form or arrest the nanoparticles in glass. Structurally, glass is an amorphous solid, lacking long range periodic arrangement as well as symmetry arrangement of atoms/molecules. When a liquid is cooled below certain temperature, it forms either a crystalline or amorphous solid (glass). Nuclei are formed spontaneously with homogenous (in the melt) or inhomogeneous (on the surface of other materials) nucleation, which can grow to form ordered, crystalline solid. Usually, metals form crystalline solids but, if cooled at very high cooling rate, they can form amorphous solids. Such solids are known as metallic glasses. Even in such cases the atoms try to reorganize themselves into crystalline solids. Addition of elements like B, P, Si etc. helps to keep the metallic glasses in amorphous state. It is possible to form nanocrystals within metallic glasses. It is also possible to form some nanoparticles by mixing the molten streams of metals at high velocity with turbulence. On mixing thoroughly, nanoparticles are formed.

(c) Physical Vapor Deposition: It involves material for evaporation, an inert gas or reactive gas for collision of material vapor, a cold finger on which clusters or nanoparticles can condense, a scraper to scrape the nanoparticles and piston- anvil (an arrangement in which nanoparticle powder can be compacted). All the processes are carried out in a vacuum chamber so that the desired purity of the end product can be obtained.

Metals or high vapor pressure metal oxides are evaporated or sublimated from filaments or boats of refractory metals like W, Ta, Mo in which materials to be evaporated are held. Size, shape and even the

phase of evaporated material can depend upon the gas pressure in deposition chamber. Clusters or nanoparticles condensed on the cold finger (water or liquid nitrogen cooled) can be scraped off inside the vacuum system. The process of evaporation and condensation can be repeated several times until enough quantity of material falls through a funnel in which a piston-anvil arrangement has been provided.

(d) Ionized Cluster Beam Deposition: It is useful to obtain adherent and high quality single crystalline thin films. The set up consists of a source of evaporation, a nozzle through which material can expand into the chamber, an electron beam to ionize the clusters, an arrangement to accelerate the clusters and a substrate on which nanoparticle film can be deposited, all housed in a suitable vacuum chamber. Small clusters from molten material are expanded through the fine nozzle. The vapor pressure, ~10 torr to 10^{-2} torr needs to be created in the source and the nozzle needs to have a diameter larger than the mean free path of atoms or molecules in vapor form in the source to form the clusters. On collision with electron beam clusters get ionized. Due to applied accelerating voltage, the clusters are directed towards the substrate. By controlling the accelerating voltage, it is possible to control the energy with which the clusters hit the substrate. Thus it is possible to obtain the films of nanocrystalline material using ionized cluster beam.

(e) Laser Vaporization: In this method, vaporization of the material is effected using pulses of laser beam of high power. The set up is a ultra high vacuum or high vacuum system equipped with inert or reactive gas introduction facility, laser beam, solid target and cooled substrate. Clusters of any material of which solid target can be made are possible to synthesize. Usually laser giving UV wavelength such as excimer laser is necessary because other wavelengths like IR or visible are often reflected by some of the metal surface. A powerful beam of laser evaporates the atoms from a solid source, atoms collide with inert gas atoms (or reactive gases) and cool on them forming clusters. They condense on the cooled substrate. The method is often known as laser ablation. Gas pressure is very critical in determining the particle size and distribution. Simultaneous evaporation of another material and mixing the two evaporated materials in inert gas leads to the formation of alloys or compounds.

(f) Laser Pyrolysis or Laser Assisted Deposition: Here a mixture of reactant gases is decomposed using a powerful laser beam in presence of some inert gas like helium or argon. Atoms or molecules of decomposed reactant gases collide with inert gas atoms and interact with each other, grow and are then get deposited on cooled substrate. Many materials like Al_2O_3 , WC, Si_3Ni_4 etc. are synthesized in nanocrystalline form by this method. Here too, gas pressure plays an important role in deciding the particle size and their distribution.

(g) Sputter Deposition: In sputter deposition, some inert gas ions like Ar are incident on a target at a high energy. The ions become neutral at the surface but due to their energy, incident ions may get implanted, get bounded back, create collision cascades in target atoms, displace some of the atoms in the target creating vacancies, interstitials and other defects, desorb some adsorbents, create photons while losing energy to target atoms or even sputter out some target atoms/molecules, clusters, ions and secondary electrons. Sputter deposition is a widely used thin film deposition technique, specially to obtain stoichiometric thin films from target material. Target material may be some alloy, ceramic or compound. It is a very good technique to deposit multilayer films for mirrors or magnetic films for spintronic applications. Sputter deposition can be carried out using Direct Current (DC) sputtering, Radio Frequency (RF) sputtering or magnetron sputtering. In all these methods, one uses discharge or plasma of some inert gas atoms or reactive gases. The deposition is carried out in a required gas pressurized high vacuum or ultra high vacuum system equipped with electrodes, one of which is a sputter target and the other is a substrate, gas introduction facility etc.

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In DC sputtering, the target is held at high negative voltage and substrate may be at positive, ground or floating potential. Substrates may be simultaneously heated or cooled depending upon the requirement. Once the required base pressure is attained in the vacuum system, usually argon gas introduced at a low pressure. A visible glow is observed and current flows between anode and cathode indicating the deposition onset. When sufficiently high voltage is applied between anode and cathode with a gas in it, a glow discharge is set up with different regions as cathode glow, Crooke's dark space, negative glow, Faraday dark space, positive column, anode dark space and anode glow. These regions are the result of plasma. Plasma is a mixture of free electrons, ions and photons. Plasma is overall neutral but there can be regions, which are predominantly of positive or negative charge. The density of various particles and the length over which they are spread and distributed depends upon the gas pressure.

In RF sputtering 5-30 MHz frequency is used and the electrodes can be insulating. However, 13.56 MHz is a commonly used frequency for deposition. Target itself biases to negative potential becoming cathode.

RF and DC sputtering efficiency can be further increased using magnetic field. When both electric and magnetic fields act simultaneously on a charged particle, force is acted upon it. Electrons moves in a helical path and is able to ionize more atoms in the gas. In practice, both parallel and magnetic fields to the direction of electric field are used to further increase the ionization of the gas, increasing the efficiency of sputtering. By introducing gases like O₂, N₂, NH₃, CH₄, H₂S etc. while metal targets are sputtered, one can obtain metal oxides like Al₂O₃, nitrides, carbides etc., This is known as reactive sputtering.

The plasma density can be further enhanced using microwave frequency and coupling the resonance frequency of electrons in magnetic field. Ionization density using Electron Cyclotron Resonance plasma is about 2-3 orders of magnitude larger. Thin films and nanoparticles of Si₂O₃, SiN, GaN etc. have been obtained using this technique.

(h) Chemical Vapour Deposition (CVD): It is a hybrid method using chemicals in vapour phase. Basic CVD process can be considered as a transport of reactant vapour or reactant gas towards the substrate kept at some high temperature where the reactant cracks into different products which diffuse on the surface, undergo some chemical reaction at appropriate site, nucleate and grow to form the desired material film. The by-products created on the substrate have to be transported back to the gaseous phase removing them from the substrate. Vapours of desired material may be often pumped into reaction chamber using some carrier gas. In some cases the reactions may occur through aerosol formation in gas phase. There are various processes such as reduction of gas, chemical reaction between different source gases, oxidation or some disproportionate reaction by which CVD can proceed. However, it is preferable that the reaction occurs at the substrate rather than in the gas phase. Usually temperature ~ 300 to 1200 C is used at the substrate. There are two ways viz., hot wall and cold wall by which substrates are heated. In hot wall set up the deposition can take place even on reactor walls. This is avoided in cold wall design. Besides this, the reaction can take place in gas phase with hot wall design, which is suppressed in cold wall set up. Further, coupling of plasma with chemical reaction in cold wall set up is feasible. Usually gas pressures in the range of 0.1 torr to 1.0 torr are used. Growth rate and film quality depend upon the gas pressure and the substrate temperature. When the growth takes place at low temperature, it is limited by the kinetics of surface tension.

CVD is widely used in industry because of relatively simple instrumentation, ease of processing, possibility of depositing different types of materials and economic viability. Under certain deposition conditions nanocrystalline films or single crystalline films are possible. There are many variants of CVD like metallo organic CVD (MOCVD), atomic layer epitaxy (ALE), vapor phase epitaxy (VPE), plasma

enhanced CVD (PECVD) etc. They differ in source gas pressure, geometrical layout, temperature used etc.

(i) Electric Arc Deposition: This is one of the simplest and useful methods, which leads to mass scale production of fullerenes and carbon nanotubes. It requires water cooled vacuum chamber and electrodes to strike an arc between them. The positive electrode itself acts as the source of material. If some catalyst are to be used, there can be some additional thermal source of evaporation. Inert gas or reactive gas introduction is necessary. Usually the gap between the electrodes is ~1mm and high current ~50 to 100 amperes is passed from a low voltage power supply (~12-15 volts). Inert gas pressure is maintained in the vacuum system. When an arc is set up, anode material evaporates. This is possible as long as the discharge can be maintained. By striking the arc between the two graphite electrodes, it is possible to get fullerenes in large quantity. In case of fullerenes, the formation occurs at low helium pressure as compared to that used for nanotube formation. Also, fullerenes are obtained by purification of soot collected from inner walls of vacuum chamber, whereas nanotubes are found to be formed only at high He gas pressure and in the central portion of the cathode. No carbon nanotubes are found on the chamber walls

(j) Ion Implantation: In this method high energy (few keV to hundreds of keV) or low energy (<200 eV) ions are used to obtain nanoparticles. Ions of interest are usually formed using an ion gun specially designed to produce metal ions, which are accelerated to high or low energy towards the substrate heated to few hundred of C. Depending upon the energy of the incident ions, various other processes like sputtering and generation of electromagnetic radiation may take place. It is possible to obtain single element nanoparticles or compounds and alloys of more than one element. In some experiments it has been possible to even obtain doped nanoparticles using ion implantation. There is possibility of making nanoparticles using swift heavy ions (few MeV energy) employing ion accelerators like a pelletron.

(k) Molecular beam epitaxy (MBE): This technique of deposition can be used to deposit elemental or compound quantum dots, quantum wells, quantum wires in a very controlled manner. High degree of purity in materials is achievable using ultra high vacuum (better than torr). Special sources of deposition known as Knudsen cell (K-cell) or effusion cell are employed to obtain molecular beams of the constituent elements. The rate of deposition is kept very low and substrate temperature is rather high in order to achieve sufficient mobility of the elements on the substrate and layer by layer growth to obtain nanostructures.

(l) Thermolysis: Nanoparticles can be made by decomposing solids at high temperature having metal cations, and molecular anions or metal organic compounds. The process is called thermolysis. For example, small lithium particles can be made by decomposing lithium azide, LiN_3 . The material is placed in an evacuated quartz tube and heated to 400 C. At about 370 C LiN_3 decomposes, releasing N_2 gas, which is observed by an increase in the pressure on the vacuum gauge. In a few minutes the pressure drops back to its original low value, indicating that all the N_2 has been removed. The remaining lithium atoms coalesce to form small colloidal metal particles. Particles less than 5nm can be made by this method. Passivation can be achieved by introducing an appropriate gas.

(m) Pulsed laser method: Pulsed lasers have been used in the synthesis of nanoparticles of silver. Silver nitrate solution and a reducing agent are flowed through a blenderlike device. In the blender there is a solid disk, which rotates in the solution. The solid disk is subjected to pulses from a laser beam creating hot spots on the surface of the disk. Silver nitrate and the reducing agent react at these hot spots, resulting in the formation of small silver particles, which can be separated from the solution using a centrifuge. The size of particles is controlled by the energy of the laser and rotation speed of the disk. This method is capable of a high rate of production.

3. Chemical Methods (Wet Chemical route)

There are numerous advantages of using chemical methods, which are –

- Inexpensive, less instrumentation compared to many physical methods
- Low temperature (< 350 C) synthesis
- Doping of foreign atoms (ions) possible during synthesis
- Variety of size and shapes are possible
- Self assembly or patterning is possible

(a) Colloids and Colloids in solutions: A class of materials in which two or more phases (solid, liquid, gas) of same or different materials co-exist with at least one dimension less than a micrometer is known as colloids. Colloids may be particles, plates, or fibers. Nanomaterials are a sub-class of colloids, in which one of the dimensions of colloids is in about 1 to 100 nm range. Colloids are the particles suspended in some host matrix.

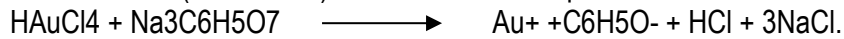
Interactions: Colloids are particles with large surface to volume ratio. Therefore atoms on the surface are in a highly reactive state, which easily interact to form bigger particles or tend to coagulate. It is thus necessary to understand the stability of colloids i.e., how the colloids dispersed in a medium can remain suspended particles. In general there are a number of interactions involved. There are two types of interactions: attractive and repulsive. Repulsive interaction involves short distance of Born repulsive interaction and long range attractive interaction van der Waals attraction. Repulsive part arises due to repulsion between electron clouds in each atom and attractive part is due to interaction between fluctuating or permanent dipoles of atoms/molecules. The attractive forces between colloidal particles reduced in colloids in a liquid medium. Colloids in liquid may be positively charged, negatively charged or even neutral. But in most cases they are charged. As there are some charges on particles, ions of opposite charges accumulate around them. Oppositely charged ions are known as counter ions. This accumulation of counter ions leads to formation of an electric double layer. Stability of colloids can be increased by steric hinderance or repulsion. By adsorbing some layers of a different material on colloidal particles eg. polymer it is possible to reduce the attractive forces between them..

Synthesis: Chemical reactions in which colloidal particles are obtained are carried out in glass reactor of suitable size. Glass reactor usually has a provision to introduce some precursors, gases as well as measure temperature, pH etc. during the reaction. It is usually possible to remove the products at suitable time intervals. Reaction is usually carried out under inert atmosphere like argon or nitrogen gas so as to avoid any uncontrolled oxidation of the products. There is also provision made to stir the reactants during the reaction by using Teflon coated magnetic needle.

Although chemical synthesis of nanoparticles is a complex process, by understanding how nucleation and growth of particles takes place, it is possible to control the various steps and try to achieve monodispersed nanoparticles. This can be done with the help of LaMer diagram. As we keep on increasing the concentration of the reactants in the solution, at certain concentration, say C_0 , the formation of nuclei begins. There is no precipitate at this concentration. Further increase in concentration increases nuclei formation up to a concentration C_N , above which there is 'super saturation' between C_N and C_S . Concentration C_N denotes the maximum rate of nuclei formation. When nuclei formation reduces, again C_0 the minimum concentration for nucleation is reached. No new nuclei can be formed and crystal growth reduces the concentration. At this concentration C_S , an equilibrium is obtained. If new nuclei are formed during the growth of particles, particle with large size distribution are obtained. Therefore it is very important that concentration of solute and its diffusion to dissolve species be adjusted properly in order that no fresh nuclei are formed once the concentration of solute and its diffusion to dissolve species be adjusted properly in order that no fresh nuclei are formed once the

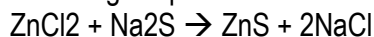
concentration has reached C_N . Particles can grow even at the expense of smaller particles. Larger particles are more stable and grow at the expense of smaller particles. This growth mode is known as Ostwald ripening. The driving force for large particles is the reduction in surface free energy.

Colloidal metal nanoparticles are often synthesized by reduction of some metal salt or acid. For example highly stable gold particles can be obtained by reducing chloroauric acid (HAuCl_4) with tri sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$). The reaction takes place as follows –

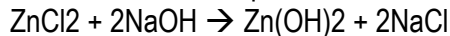


Au atoms are formed by nucleation and condensation. They grow bigger in size by reduction of more Au^+ ions on the surface. These atoms are stabilized by oppositely charged citrate ions. Metal gold nanoparticles exhibit intense red, magenta etc., colours, depending upon the particle size. Gold nanoparticles are stabilized by repulsive Coulomb interaction. It is also possible to stabilize gold nanoparticles using thiol or some other capping molecules. In a similar manner, silver, palladium, copper and other metal nanoparticles can be synthesized using appropriate precursors, temperature, pH, duration of synthesis etc., Particle size, size distribution and shape strongly depend on the reaction parameters and can be controlled to achieve desired results. It is also possible to synthesize alloy nanoparticles using appropriate precursors.

Compound semiconductor nanoparticles can be synthesized by wet chemical route using appropriate salts. Sulphide semiconductors like CdS and ZnS can be synthesized easily by what is known as co-precipitation. For example to obtain ZnS nanoparticles any zinc salt like Zinc sulphate (ZnSO_4), zinc chloride (ZnCl_2) can be dissolved in aqueous (or nonaqueous) liquid and Na_2S is added to the solution. Following simple reaction results to give particles of ZnS.



To obtain zinc oxide particles one can use following reactions:



Selenide particles can be obtained using appropriate selenium giving salt. However, all these nanoparticles need to be surface passivated as colloids formed in liquids have a tendency to coagulate or ripen due to attractive forces existing between them. The electrostatic and other repulsive forces may not be sufficient to keep them apart. However, steric hindrance can be created by appropriately coating the particles to keep them apart. This is often known as 'chemical capping' and has become a widely used method in the synthesis of nanoparticles. Advantage with this chemical route is that, one can get stable particles of variety of materials not only in the solution, but even after drying off the liquid. Coatings may be part of post-treatment or a part of the synthesis reactions to obtain nanoparticles. If it is a part of the synthesis reaction, the concentration of capping molecules can be used in two ways, to control the size as well as to protect the particles from coagulation. Chemical capping can be carried out at high or low temperature depending on the reactants. In high temperature reactions, cold organometallic reactants are injected in some solvent like triocylphosphineoxide held at temperature $>300^\circ\text{C}$.

(b) Langmuir-Blodgett (L-B) method: This technique to transfer organic layers at air-liquid interface onto solid substrates is known for nearly 70 years. The technique was developed by the two scientists Langmuir and Blodgett. In this technique one uses amphiphilic long chain molecules like that in fatty acids. An amphiphilic molecule has a hydrophilic group (water loving) at one end and a hydrophobic group (water hating) at the other end. As an example consider the molecule of arachidic acid, which has a chemical formula $[\text{CH}_3(\text{CH}_2)_{16}\text{COOH}]$. There are many such long chain organic chains with general chemical formula $[\text{CH}_3(\text{CH}_2)_n\text{COOH}]$, where n is a positive integer. In this case, $-\text{CH}_3$ is hydrophobic and $-\text{COOH}$ is hydrophilic in nature.

Usually molecules with $n > 14$ are candidates to form L-B films. This is necessary in order to keep hydrophobic and hydrophilic ends well separated from each other. When such molecules are put in water, the molecules spread themselves on surface of water in such a way that their hydrophilic ends, often called as heads, are immersed in water, whereas the hydrophobic ends called as tails remain in air. They are also surface active agents or surfactants. Surfactants are amphiphilic molecules i.e. an organic chain molecule in which at one end there is polar, hydrophilic (water loving) and at the other a nonpolar, hydrophobic (water hating) group of atoms. Using a movable barrier, it is possible to compress these molecules to come close together to form a monolayer and align the tails. It is however necessary that hydrophilic and hydrophobic ends are well separated. Such a monolayer is two dimensionally ordered and can be transferred on some suitable solid substrates like glass, silicon etc. This is done by dipping the solid substrate in the liquid, in which ordered organic molecular monolayer is already formed.

Deposition of L-B films is done by following steps: (1) A monolayer of amphiphilic molecules is formed (2) A substrate is dipped in the liquid (3) The substrate is pulled out, during which ordered molecules get attached to the substrate (4) When the substrate is again dipped, molecules again get deposited as the substrate forming a second layer on the substrate (5) As the substrate is again pulled out a thin layer gets deposited. By repeating the procedure large number of ordered layers can be transformed on a substrate.

In general there are three types of L-B films with different multilayer sequence. These are known as X, Y, and Z type. (1) X-type: Deposition only during insertion of substrate (2) Y-type: Deposition both the times except no deposition during first immersion (3) Z-type: Deposition only during removal of substrate. Y type of films are most common. Although the layers are ordered, there is only the van der Waals interaction between different layers. Thus L-B films are good examples of nanostructured materials.

It is possible to obtain nanoparticles using L-B technique. A metal salt like CdCl_2 or ZnCl_2 is dissolved in water on surface of which a compressed uniform monolayer of surfactant is spread. When H_2S gas is passed in the solution, CdS or ZnS nanoparticles of few tens of nanometers can be formed. Particles are uniform in size. If surfactants are not present, uniform nanoparticles are not formed.

(c) Sol-Gel Method: As the name implies sol-gel involves two types of materials or components 'sol' and 'gel'. There are several advantages of sol-gel: All sol-gel formation process is usually a low temperature process. This means less energy consumption and less pollution too. Some of the benefits like getting unique materials such as aerogels, zeolites, ordered porous solids by organic-inorganic hybridization are unique to sol-gel process. It is also possible to synthesize nanoparticles, nanorods, nanotubes etc., using sol-gel technique.

Sols are solid particles in a liquid. They are thus a subclass of colloids. Gels are nothing but a continuous network of particles with pores filled with liquid (or polymers containing liquid). A sol-gel process involves formation of 'sols' in a liquid and then connecting the sol particles (or some subunit capable of forming a porous network) to form a network. By drying the liquid, it is possible to obtain powders, thin films or even monolithic solid.

Synthesis of sol-gel in general involves hydrolysis of precursors, condensation followed by polycondensation to form particles, gelation and drying process by various routes. Precursors (starting chemicals) are to be chosen so that they have a tendency to form gels. Both alkoxides or metal salts can be used. Alkoxides have a general formula $\text{M}(\text{ROH})_n$, where M is a cation, R an alcohol group, and n is the number of (ROH) groups with each cation. Salts are denoted as MX, in which M is a cation and X is an anion. Although it is not mandatory that only oxides be formed by a sol-gel process, often oxide ceramics are best synthesized by a sol-gel route. For example in silica, SiO_4 group with Si at the centre and four oxygen atoms at the apexes of tetrahedron are very ideal for forming sols with interconnectivity through the corners of tetrahedrons, creating some cavities or pores. By polycondensation process (i.e., many hydrolyzed units coming together by removal of some atoms from small molecules like OH) sols

are nucleated and ultimately sol-gel is formed. Sol-gel method is particularly useful to synthesize ceramics or metal oxides although sulphides, borides and nitrides also are possible.

(d) Microemulsion: Synthesis of nanoparticles in the cavities produced in microemulsion is a widely used method. Advantage of this method is the biocompatibility and biodegradability of synthesized materials. Biocompatibility is useful in drug delivery of nanomaterials and biodegradability is environmentally useful. Whenever two immiscible liquids are mechanically agitated or stirred together, they are known to form what is called 'emulsion'. The tendency of the liquids is such that the liquid is smaller quantity tries to form small droplets, coagulated droplets or layers so that they are all separated from the rest of the liquid (for example droplets of fat in milk). The droplet sizes in emulsion are usually larger than 100 nm upto even few millimeters. Emulsions are usually turbid in appearance. On the other hand, there is another class of immiscible liquids, known as microemulsions which are transparent and the droplets are in the range of ~1 to 100 nm. This is size needed for the synthesis of nanomaterials. Microemulsions are stabilized using surfactants (surface stabilized active agents). When an organic liquid or oil (O), water (W) and surfactant (T) are mixed together, under some critical concentration, 'micelles' or inverse micelles are formed, depending upon the concentration of water and organic liquid. Micelles are formed with excess water and inverse micelles are formed in excess of organic liquid or oil. The ratio of water, oil and surfactant is important to decide which type of micelle will be formed and can be represented in a ternary phase diagram, using a triangle. Composition can be determined by drawing lines parallel to all three sides of the triangle. A modified phase diagram known as 'Winsor Diagram' also can be constructed for finer details. The critical micelle concentration (CMC) depends upon all W, O and T concentrations. Effect of T is to reduce the surface tension of water dramatically below CMC and remain constant above it, as the organic solvent concentration is kept on increasing. There are four types of surfactants in general:

Cationic: eg. CTAB

Anionic: eg. R-SO₃-Na⁺

Nonionic: R-(CH₂-CH₂-O)₂₀-H

Amphoeric: eg. betaines.

A large number of nanoparticles of (metals, semiconductors and insulators) cobalt, copper, CaCO₃, BaSO₄, CdS, ZnS etc, have been synthesized using microemulsions or inverse micelles. Eg. synthesis of cobalt nanoparticles – A reverse micellar solution of water and oil can be stabilized using a monolayer of surfactant like sodium bis (2-ethylhexyl) sulfosuccinate or Na(AOT). The droplet diameter is controlled simply by controlling the amount of water. Two micellar solutions having same diameter of droplets can be formed. Thus one solution should have Co(AOT)₂ i.e., cobalt bis (2-ethylhexyl) sulfosuccinate and the other should have sodium tetrahydroborate (NaBH₄). When two solutions are mixed together the solution appears clear but the color changes from pink to black. One can find by electron microscopy analysis that cobalt nanoparticles are formed.

(e) Other Chemical Methods: Several types of reducing agents can be used to produce nanoparticles such as NaBEt₃H, LiBEt₃H, and NaBH₄ where Et denotes ethyl (-C₂H₅) radical. For example, nanoparticles of molybdenum (Mo) can be reduced in toluene solution with NaBEt₃H at room temperature, providing a high yield of Mo nanoparticles having dimensions of 1-5 nm.

Nanoparticles of aluminum have been made by decomposing Me₂EtAlH₃ in toluene and heating the solution to 105 C for 2 h (Me is methyl, -CH₃). Titanium isopropoxide is added to the solution. The titanium acts as a catalyst for the reaction. The choice of catalyst determines the size of the particles produced. For instance, 80 nm particles have been made using titanium. A surfactant such as oleic acid can be added to the solution to coat the particles and prevent aggregation.

4. Biological Methods

Synthesis of nanomaterials using biological ingredients can be roughly divided into following three types:

- use of microorganisms
- use of enzymes or plant extracts
- use of templates like DNA, membranes, viruses

(a) Synthesis using microorganisms: Microorganisms are capable of interacting with metals coming in contact with them through their cells and form nanoparticles. Different processes of metal-microorganism interactions are: (i) Some microorganisms produce hydrogen sulfide (H₂S). It can oxidize organic matter forming sulphate, which in turn acts like an electron acceptor for metabolism. This H₂S can, in presence of metal salt, convert metal ions into metal sulphide, which deposits extracellularly. (ii) In some cases, metal ions from a metal salt enter the cell. The metal ions are then converted into a nontoxic form and covered with proteins in order to protect the remainder of cell from toxic environment. (iii) certain microorganisms are capable of secreting some polymeric materials like polysaccharides. They have some phosphate, hydroxyl and carboxyl anionic groups which complex with metal ions and bind extracellularly (iv) cells are also capable of reacting with metals or ions by processes like oxidation, reduction, methylation, demethylation etc.

Examples:

- *Pseudomonas stutzeri* Ag259 bacteria are found in silver mines and are capable of accumulating silver inside or outside of their cells walls. Using this fact these bacterial strains can be challenged with high concentration of silver salt like AgNO₃. Numerous silver nanoparticles of different shapes can be produced having size <200 nm intracellularly.
- Low concentrations of metal ions (Au⁺, Ag⁺ etc.) can be converted to metal nanoparticles by *Lactobacillus* strain present in butter milk. By exposing the mixture of two different metal salts to bacteria, it is indeed possible to obtain alloys under certain conditions.
- *Fusarium oxysporum* challenged with gold or silver salt for approximately three days produces gold or silver particles extracellularly.. Extremophilic actinomycete *Thermomonospora* sp. produces gold nanoparticles extracellularly.
- When silver metal salt is treated with fungus *Verticillium* sp. the nanoparticles can be produced intracellularly. Changes in biomass colour from initial yellow to final brown, after exposure to silver salt, is a visual indication of silver nanoparticles formation. Particles can be recovered by washing with some suitable detergent or ultrasonication. In a similar way, gold nanoparticles can be produced using *Verticillium* sp. However, the colour of biomass is from pink to blue depending upon the particle size.
- Semiconductor nanoparticles like CdS, ZnS, PbS etc. can be produced using different microbial routes. *Desulfobacteriaceae* can form 2-5 nm ZnS nanoparticles. Bacteria *Klebsilla pneumoniae* can be used to synthesize CdS nanoparticles. When Cd(NO₃)₂ is mixed in a solution containing bacteria and solution is shaken for about one day at ~38 C, then the CdS nanoparticles in the size range ~5-200 nm can be formed. CdS nanoparticles with narrow size distribution can be synthesized using the yeasts like *Candida glabrata* and *Schizosaccharomyces pombe*. Similarly it is possible to synthesize PbS by challenging *Torulopsis* sp. with lead salt like PbNO₃.

(b) Synthesis using plant extracts: It has been reported that live alfalfa plants are found to produce gold nanoparticles from solids. Leaves from geranium plant (*pelargonium graveolens*) have also been used to synthesize nanoparticles of gold. Nanoparticles obtained using *Collectotrichum* sp. Fungus related to geranium plant has a wide distribution of sizes and particles are mostly spherical. On the other hand, geranium leaves produce rod and disk shaped nanoparticles. Synthesis procedure to obtain

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gold nanoparticles from geranium plant extract is as follows: Finely crushed leaves are put in Erlenmeyer flask and boiled in water just for a minute. Leaves get ruptured and cells release intracellular material. Solution is cooled and decanted. This solution is added to HAuCl_4 aqueous solution and nanoparticles of gold start forming within a minute.

(c) Use of templates: DNA, S-layers or some membranes have long range periodic order in terms of some molecular groups of their constituents. Therefore on some periodic active sites preformed nanoparticles can be anchored. Alternatively, using certain protocols nanoparticles can be synthesized using DNA, membranes etc., as templates. Such ordered arrays are formed as a result of various interactions that take place between the templates and the particles.

Ferritin is a colloidal protein of nanosize. It stores iron in metabolic process and is abundant in animals. It is also capable of forming uniform three dimensional hierarchical architecture. There are 24 protein (peptides) subunits in a ferritin, which are arranged in such a way that they create a central cavity of ~6nm. Diameter of polypeptide shell is 12 nm. Ferritin can accommodate 4500 Fe atoms. They are in Fe^{3+} state as hydrated iron oxide mineral, ferrihydrite. The protein subunits are composed of light as well as heavy chains having dinuclear ferroxide centres. These centres are catalysts for in vitro oxidation of Fe^{2+} ions. The ferritin without inorganic matter in its cavity is known as apoferritin and can be used to entrap desired nanomaterial inside the protein cage. Therefore, first step is to remove iron from ferritin to form apoferritin and then introduce metal ions to form metal nanoparticles inside the cavity or carry out some controlled reaction with metal ions to make a compound inside the cavity. In any case, ions can be removed or introduced inside the ferritin, through some available channels.

Horse spleen ferritin, diluted with sodium acetate buffer, should be placed in dialysis bag. A solution of sodium acetate and thioglycolic acid is made in which dialysis bag is kept under nitrogen gas flow for 2-3 hours. Solution needs to be replaced from time to time for total 4-5 hours. Further dialysis of apoferritin solution should be done against saline for one hour and in refreshed saline for ~15-20 hours. Apoferriting should then be mixed with solution having sodium chloride (NaCl) and N-tris (hydroxymethyl) methyl-2-aminoethanosuphonic acid (TES). Aqueous cadmium acetate is added to this solution and stirred continuously with constant N_2 gas purging. Process of CdS formation is stepwise with Cd loading of 55 atoms per apoferritin colloid taking place in each step. Higher loading like 110, 165, 220 are possible. Due to remarkably constant size of ferritin colloids and apoferritin derived from them, it is possible to obtain nanoparticles of very uniform size. Besides CdS there are several other examples like controlled iron oxide, manganese, uranyl oxide, cobalt, cobalt-platinum alloy etc., being synthesized inside ferritin. It is possible to fabricate ordered arrays of ferritin as well as of nanoparticles inside them.

DNA can be used for preformed charged nanoparticles can get bonded with phosphate group of DNA and even form organized arrays of nanoparticles. CdS (or other sulfide) nanoparticles can be synthesized using DNA. Organic molecules can cap the surfaces of nanoparticles growing in solutions. Similarly one can use DNA to bind with surface of growing nanoparticles. For example, double stranded Salmon sperm DNA can be sheared to an average size of 500 bp. Cadmium acetate can be added to desired medium like water, dimethylformamide, ethanol, propanol etc., and reaction carried out in a glass flask with facility to purge the solution and flow with an inert gas like nitrogen. Addition of DNA should be made and then Na_2S can be added dropwise. Depending upon the concentrations of cadmium acetate, sodium chloride and DNA nanoparticles of CdS with size less than ~10 nm can be obtained. DNA probably bonds through its negatively charged phosphate group to positively charged (Cd^+) nanoparticle surface.