CONT'D

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 stearic 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..

Syntheis: 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_O, 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_O 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 choloroauric acid (HAuCl4) with tri sodium citrate ($Na_3C_6H_5O_7$). The reaction takes place as follows –

$$\text{HAuCl4} + \text{Na3C6H5O7} \longrightarrow \text{Au+} + \text{C6H5O-} + \text{HCl} + 3\text{NaCl}.$$

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 (ZnSO4), zinc chloride (ZnCl2) can be dissolved in aqeous (or nonaqeous) liquid and Na2S is added to the solution. Following simple reaction results to give particles of ZnS.

$ZnCl2 + Na2S \rightarrow ZnS + 2NaCl$

To obtain zinc oxide particles one can use following reactions:

$ZnCl2 + 2NaOH \rightarrow Zn(OH)2 + 2NaCl$

$Zn(OH)2 \rightarrow ZnO + H2O$

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, stearic 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 aterials 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 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 ahs a chemical formula [CH3(CH2)16 COOH]. There are many such long chain organic chains with general chemical formula [CH3(CH2)nCOOH], where n is a positive integer. In this case, -CH3 is hydrophobic and –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 CdCl2 or ZnCl2 is dissolved in water on surface of which a compressed uniform monolayer of surfactant is spread. When H2S 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 M(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, SiO4 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 polycodensation process (i.e., many hydrolyzed units coming together by removal of some atoms from small molecules like OH) sols are nucleated and ultimately solgel 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. Biocompatability 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 miceller solution of water and oil can be stabilized using a monlayer 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 shuld 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 Me2EtNAlH3 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 extracellulary. (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 bateria 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 shaked 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 gold nanoparticles form 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 architechture. 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 2-3hours. 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 ae 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 Na2S 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.