SIMPLIFIED PRACTICALS ON THE GREEN SYNTHESIS OF NANOPARTICLES

Preamble

The green approach in the synthesis of nanoparticles, which precludes the use of hazardous procedures and chemicals, has contributed to the expansion of applications of nanoparticles for the production of biocompatible and eco-friendly particles using low-cost and benign approach. Furthermore, the abundance of biomolecules in diverse biological entities such as plants, microbes, agro wastes, pigments, enzymes, arthropods and their metabolites have also added to the growing trend in the green and one-pot synthesis of nanoparticles for diverse applications.

Sliver nanoparticles

Silver nanoparticles (AgNPs) have been widely studied for their numerous and excellent properties and applications. These include optical, bio-imaging, catalytic, antiplatelet, anticoagulant, thrombolytic, fibrinolytic, sensing, wound-healing, larvicidal, antimicrobial, anti-helminth, anti-diabetic, anti-inflammatory, anti-protozoan, antioxidant, biodesulphurization and anticancer applications.

Gold nanoparticles

Gold nanoparticles (AuNPs) have been known to exhibit considerable biocompatibility, in view of the fact that gold is not readily oxidized unlike silver. Therefore, it has potential to be used for long-term biomedical applications as it displays low-toxicity. It has been reported that conjugation of AuNPs with antibodies and proteins enhance their functionality for sensing and therapeutic functions. AuNPs have been studied for different types of applications including catalytic, bioimaging, antioxidant, photothermal, anticancer, anticoagulant, fluorescent, biolabelling, biosensing, antimicrobial, and thrombolytic purposes

Silver-gold alloy nanoparticles

Bimetallic nanoparticles have gained attentions in their synthesis and applications, owning to the fact that they combine attributes of the monometallic components and by altering the molar ratios of the two metals. Unique bimetallic nanoparticles can be created with very good properties for diverse applications. Amongst such bimetallic nanoparticles of importance is Ag-AuNPs, which have been synthesized using the biological route. Ag-AuNPs with a single surface plasmon resonance (SPR) band located at an intermediate position between the SPR band of monometallic Au and Ag nanoparticles, may have lower toxicity compared to AgNPs, thereby enhancing the biocompatibility for biomedical applications. Unlike Ag and AuNPs, the reports on biomedical applications of green Ag-AuNPs are scanty, thereby necessitating intensive investigations on the potentials of the bimetallic material.

Practical One

Green synthesis of silver nanoparticles using the pod extract of *Cola nitida*

Aim: To synthesize silver nanoparticles using the pod extract of *Cola nitida.*

Objective: To demonstrate that biological materials, which are rich in several organic compounds can be used for the biofabrication of silver nanoparticles under benign conditions.

Learning outcome: At the end of this practical session, participants should have acquired adequate knowledge for the green synthesis of silver nanoparticles, and should be able to demonstrate same.

Procedure

Materials/equipment: Fresh pod of *C. nitida*, grinder/blender, water bath or hot plate, distilled water, filter, centrifuge, pipette, silver nitrate solution (1 mM), spectrophotometer, FTIR, TEM, XRD, DLS.

Activities

i. Collect matured fresh fruit of *C. nitida*, remove the seeds and chop the pod into pieces and air-dry at room temperature.

ii. Grind the dried pod chips into powder, and store in air-tight container.

iii. Extract the pod powder (0.1 g in 100 ml of water) using hot water at 60 ^{o}C for 1 h, after which the extract is allowed to cool, filtered using Whatman No. 1 filter paper and further centrifuged at 4000 rpm for 15 min to obtain clear extract.

iv. Prepare 1 mM $AgNO_3$, and keep away from sunlight.

v. Set-up two reaction bottles containing 40 ml of 1 mM $AgNO_3$, then add 1 ml of pod extract to bottle A (experimental), while bottle B is left to contain only $AgNO_3$. A third bottle may be set-up to contain only the pod extract.

vi. After (v) above, observe the development of change in colour in the three bottles, noting the time of onset of colour development and the stabilization of the colour. Endeavour to take fine photographs of the set-ups as colour development progresses.

Observation

i. Development of colour in bottle B only is an indication of formation of AgNPs, orchestrated by phytochemicals in pod extract that are acting as both bioreduction and capping molecules as follows:

$$Ag^{+} + e^{-} \rightleftharpoons Ag^{0} \qquad \text{(Eq. 1)}$$

ii. The rate of development of colour, the nature of colour produced and the intensity can be influenced by the type and richness of phytochemicals in the extract, which in turn influence the SPR, size, dispersity, agglomeration or stabilization and shape of the nanoparticles.

Characterization

i. Scan the absorbance of the contents of the bottles on spectrophotometer (190-900 nm).
ii. Obtain the FTIR spectra of the nanoparticles and pod extract (4000-400 cm^{-1}).
iii. Use TEM to analyze the nanoparticles to obtain images, SAED and EDX patterns.
iv. Analyze your samples using XRD, DLS, and TGA.

Exercise

i. Participants would be given solution of silver nitrate, and some known extracts of biological origin.
ii. Participants would demonstrate the synthesis of AgNPs.
iii. Participants would present and discuss their findings.

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Practical Two

Biofabrication of gold nanoparticles using the pod extract of *Theobroma cacao*

Aim: To synthesize gold nanoparticles using the pod extract of *T. cacao*

Objective: To demonstrate that biological materials, which are rich in several organic compounds can be used for the biofabrication of gold nanoparticles under benign conditions.

Learning outcome: At the end of this practical session, participants should have acquired adequate knowledge for green synthesis of gold nanoparticles, and should be able to demonstrate same.

Note: This practical would be conducted in line with practical one above, except that 1 mM $HAuCl_4$ would be used instead of $AgNO_3$ to synthesize AuNPs.

Exercise

i. Participants would be given solution of gold chloride, and some known extracts of biological origin.
ii. Participants would demonstrate the synthesis of AuNPs.
iii. Participants would present and discuss their findings.

Practical Three

Phytosynthesis of silver-gold alloy nanoparticles using the pod extract of *C. nitida*

Aim: To synthesize silver-gold nanoparticles using the pod extract of *C. nitida*.

Objective: To demonstrate that biological materials, which are rich in several organic compounds can be used for the biofabrication of silver-gold alloy nanoparticles under benign conditions.

Learning outcome: At the end of this practical session, participants should have acquired adequate knowledge for green synthesis of silver-gold alloy nanoparticles, and should be able to demonstrate same.

Note: This practical would be conducted in line with practical one above, except that 1 mM $HAuCl_4$ and $AgNO_3$ would be used in the mixture of 1:3 instead of $AgNO_3$ to synthesize Ag-AuNPs.

Exercise

i. Participants would be given mixed solution of silver nitrate and gold chloride, and some known extracts of biological origin.

ii. Participants would demonstrate the synthesis of Ag-AuNPs.

iii. Participants would present and discuss their findings.

Practical Four

Biosynthesis of zinc oxide nanoparticles (ZnONPs)

Aim: To synthesis zinc oxide nanoparticles using materials of biological origin i.e. extracts of plant (leaf, seed, stem bark, root etc), animal parts (hair or fur, insect metabolites; cob web, honey), bacteria and their metabolites

Objective: To demonstrate that biological materials, which are rich in several organic compounds can be used for the biosynthesis of zinc oxide nanoparticles under benign conditions.

Learning outcome: The participants would have acquired basic knowledge needed for green synthesis of zinc oxide nanoparticles and should be able to replicate same.

Materials/equipment: Fresh plant sample, grinder/blender, water bath or hot plate, distilled water, filter paper, centrifuge, pipette, zinc sulphate heptahydrate solution (100 mM), 1 M solution of NaOH, spectrophotometer, FTIR, TEM, XRD, DLS etc.

Procedure

i. Preparation and extraction of the biological material is carried out as explained in Practical I

ii. Prepare 100 mM $ZnSO_4(7H_2O)$ and 1 M NaOH, store and keep in clean cupboard.

iii. Measure 100 ml of 100 mM zinc sulphate heptahydrate into a 250 ml conical flask and keep stirring in a water bath equipped with shaker set at 60 ^oC. To this is added 15 ml of the biological extract in a drop wise manner until there is a change in colour (golden yellow).

iv. Check the pH of the reaction mixture and adjust to 12 by addition of 1 M NaOH. A white cloudy appearance marks the formation of ZnO nanoparticles.

v. This white solution is allowed to stand in the same condition for 2 h and later incubated overnight at room temperature. The solution is centrifuged at 5000 rpm for 20 min, white pellet is collected and dried in an oven at 150 ^{o}C.

vi. The dried pellets are collected, made into powder, collected and stored for further use.

Note: The colour of the extract may influence the degree of whiteness of the precipitate of ZnONPs.

Exercise

i. Each group of the participants would be given solution of zinc sulphate heptahydrate, 1 M NaOH and some known extracts of biological origin.

ii. Each group would demonstrate the synthesis of ZnONPs.

iii. Participants would present and discuss their findings.

Practical Five

Synthesis of biocompatible Graphene

Preamble

Graphene is the only form of carbon (generally all solid materials) in which each single atom is in exposure for chemical reactions from two sides (due to the 2D structure). This carbon nanoparticles occupy a central position in material science because of its wide applications in light weight, thin, flexible, yet durable display screen, electric circuit, electronics, solar cell, filtration, photovoltaic and energy storage as well as various medical, chemical and biological processes. The synthesis of graphene from graphite generally involves two steps: 1) conversion of graphite (Gt) to graphene oxide (GO) and 2) the reduction of GO to graphene (rGO). The second step is the most important, where strong chemicals which are known to be toxic i.e. sodium borohydride, hydrazine hydrate etc are employed as reducing agents. In this era, researchers have developed various green methods of fabricating biocompatible and eco-friendly graphene for wider application using small organic molecules and biomolecules of diverse biological origin such as plants, microbes, animals and their metabolites as reducing and stabilizing agents.

Aim: To synthesis graphene (rGO) from graphite (Gt) using materials of biological origin i.e. extracts of plant (leaf, seed, stem bark, root etc), animal parts (hair or fur, insect metabolites; cob web, honey), bacteria and their metabolites

Objective: To demonstrate that biological materials, which are rich in several organic compounds can be used for the biosynthesis of graphene under benign conditions.

Learning outcome: The participants would have acquired basic knowledge needed for green synthesis of graphene (rGO) and should be able to replicate same.

Materials/equipment: Fresh plant sample, grinder/blender, water bath or hot plate, distilled water, filter paper, centrifuge, pipette, graphite powder, H_2SO_4, $KMnO_4$, $NaNO_3$, H_2O_2, spectrophotometer, FTIR, SEM,TEM, XRD, Raman Spectra etc.

Procedure

Preparation of the extract

Preparation and extraction of the biological material is carried out as explained in Practical I

Preparation of graphene oxide (GO) from graphite

i. Add 2 g of graphite powder (Gt) to 35ml of 98% H_2SO_4 and stir on a magnetic stirrer for 2 h;
ii. To this reaction mixture, add 6 g of $KMNO_4$ gradually by maintaining the temperature below 20 ^oC;
iii. Stir the mixture at 35 ^oC for 4h in a water bath. Then, dilute the resulting solution by adding 90 ml of water under vigorous stirring for 1 h to obtain a dark brown suspension;
iv. The suspension is further treated by addition of 30% of H_2O_2 solution drop wise until the colour of the solution becomes bright yellow, indicating the oxidation pristine Gt to GtO;
v. The resulting GtO suspension is washed by repeated centrifugation, first with 5% aqueous HCl solution to remove excess of manganese salt, followed by distilled water until the pH of the solution is neutral. The sample of GtO is obtained by drying;
vi. The purified GtO is dispersed in water (0.5 mg/ml) ultrasonically for 30 min in an ultrasonic bath to obtain a stable dispersion of graphene oxide (GO).

Biofabrication of Graphene (rGO) by reduction of Graphene oxide (GO)

i. Add 10 ml of the biological extract to 90 ml of 0.5 mg/ml of aqueous GO solution and keep the mixture in a tightly sealed bottle.
ii. Stir and maintain the reaction mixture at 80 ^oC for 12 h to obtain a homogeneous green reduced GO (rGO) without aggregation.
iii. The green reduced GO is filtered and washed with hot distilled water to obtain a black G-rGO dispersion.

Exercise

i. Each group of the participants would be given sample of graphite or graphene oxide (GtO), solution of H_2SO_4, H_2O_2, HCl and salts of $KMnO_4$, $NaNO_3$, and some known extracts of biological origin.
ii. Each group would demonstrate the synthesis of rGO from graphite (Gt / GtO).
iii. Participants would present and discuss their findings.

GLOSSARY FOR TECHNICAL TERMS

Anisotropic: Anisotropic is the existence of nanoparticles with several shapes.

Anticoagulant: Anticoagulant is a material that can prevent coagulation of blood.

Antimicrobial: Antimicrobial is a phenomenon whereby growth of microbes are inhibited.

Antioxidants: Antioxidants are chemicals that have the ability to scavenge or mop up free radicals.

Atomic Force Microscope (**AFM**): An instrument able to image surfaces to molecular accuracy by mechanically probing their surface contours.

Bimetallic: Bimetallic is a nanoparticles of alloys; consisting of two chemically reacted metallic elements.

Biocompatibility: Biocompatibility is an attuned property of materials in the living cell to achieve negligible or no toxicity.

Biomedical: Biomedical is a means of both biological and medical importance.

Biomimetics: Study of the structure and function of biological substances to make artificial products that mimic the natural ones.

BioNEMS: Biofunctionalized nanoelectromechanical systems.

Bioreduction: Bioreduction is the process of reducing metal ions to their metallic states.

Biosynthesis: Biosynthesis is a process which uses the biological resource materials to synthesize nanoparticles.

Capping: Capping is the covering of surface of nanoparticles by materials to prevent aggregation or ensure functionalization.

Chemical Vapour Deposition (CVD): A technique used to deposit coatings, where chemicals are first vaporized, and then applied using an inert carrier gas such as nitrogen.

Crystal: A piece of a homogeneous solid substance having a natural geometrically regular form with symmetrically arranged plane faces.

Crystalline: Composed of crystals, or having the structure or form of a crystal.

Crystallinity: This refers to the degree of structural order in a solid.

Dendrimers: From the Greek word dendra - tree, a dendrimer is a polymer that branches.

Differential Scanning Calorimetry: It is used to analyze carrier-drug interaction.

DNA Chip: also: Gene Chip and DNA Microchip. A purpose built microchip used to identify mutations or alterations in a gene's DNA.

Dry Nanotechnology: Derives from surface science and physical chemistry, focuses on fabrication of structures in carbon (e.g. fullerenes and nanotubes), silicon, and other inorganic materials.

Dynamic Light Scattering (DLS): This technique is used to obtain the particle size distribution for the nanoparticles.

Energy-dispersive X-ray spectroscopy (EDX/EDS): With this analytical technique, elemental or chemical compositions of nanoparticles are obtained.

Facile synthesis: Non-complex, simple synthesis.

Fourier Transform Infrared (FTIR): This is a technique used to obtain an infrared spectrum of absorption or emission of a nanoparticles.

Fullerenes: Fullerenes are a molecular form of pure carbon discovered in 1985. They are cage-like structures of carbon atoms, the most abundant form produced is buckminsterfullerene (C60), with 60 carbon atoms arranged in a spherical structure.

Genetic Algorithm: Any algorithm which seeks to solve a problem by considering numerous possibilities at once, ranking them according to some standard of fitness, and then combining ("breeding") the fittest in some way. In other words, any algorithm which imitates natural selection.

Green synthesis: Green synthesis is an approach which is used for natural materials to synthesize nanoparticles under ambient conditions.

Isotropic: Isotropic is the existence of nanoparticles with a type of shape/morphology.

Larvicidal: Larvicidal is an ability to kill the larvae of insects.

LC_{50}: LC_{50} is a lethal doze that achieved 50 % death.

MEMS: **Micro Electro Mechanical Systems**: generic term to describe micron scale electrical/mechanical devices.

Microencapsulation: Individually encapsulated small particles.

Molecular Integrated Microsystems (MIMS): Microsystems in which functions found in biological and nanoscale systems are combined with manufacturable materials.

Molecular Nanotechnology (MNT): Thorough, inexpensive control of the structure of matter based on molecule-by-molecule control of products and byproducts; the products and processes of molecular manufacturing, including molecular machinery.

Monodisperse: Monodisperse is the attribute of nanoparticles in having narrow size range.

Monometallic: Monometallic is a nanoparticles of a single metal element.

Nanobarcode: Technology that uses cylindrically-shaped colloidal metal nanoparticles, in which the metal composition alternated along the length and the size of each metal segment can be controlled.

Nanobeads: Polymer beads with diameters of between 0.1 to 10 micrometers. Also called nanodots, nanocrystals and quantum beads.

Nanobiotechnology: Nanobiotechnology is an aspect of biotechnology that is concerned with creations, modifications and applications of nanomaterials to render goods and services for mankind.

Nanofabrication: Construction of items using assemblers and stock molecules. Also known as Nanofacture or nanoscale engineering.

Nanoimprinting: Sometimes called soft lithography. A technique that is very simple in concept and totally analogous to traditional mould- or form-based printing technology, but that uses moulds (masters) with nanoscale features. As with the printing press, the potential for mass production is clear. There are two forms of nanoimprinting, one that uses pressure to make indentations in the form of the mould on a surface, the other, more akin to the printing press, that relies on the application of "ink" applied to the mould to stamp a pattern on a surface. Other techniques such as etching may then follow.

Nanomaterials: can be subdivided into nanoparticles, nanofilms and nanocomposites. The focus of nanomaterials is a bottom up approach to structures and functional effects whereby the building blocks of materials are designed and assembled in controlled ways.

Nanomedicine: Nanomedicine is a field of study that deals with the application of nanotechnology in medical field.

Nanomesh and Nanofibres: (or "Nanofibers") referred to as "polymeric" (made from polymers). Currently used in air and liquid filtration applications. Using a process called "electrospinning" - or e-spin - a polymer "mesh" is formed into a nanofiber membrane, hence "nanomesh", with 150 - 200 nm diameters.

Nanoparticles: Nanoparticles are materials at nano-scale size having dimension of 1-100 nm.

Nanopharmaceuticals: nanoscale particles used to modulate drug transport for drug uptake and delivery applications.

Nanoprobe: Nanoscale machines used to diagnose, image, report on, and treat disease within the body.

Nanosensors: Nanoscale sensors.

Nanotube: A one dimensional fullerene (a convex cage of atoms with only hexagonal and/or pentagonal faces) with a cylindrical shape.

One-pot synthesis: One-pot synthesis is a scheme of synthesis, whereby bioreduction and capping of nanoparticles occur in a single step.

Polydisperse: Polydisperse is the attribute of nanoparticles in having wide size range.

Quantum Dots: nanometer-sized semiconductor crystals, or electrostatically confined electrons. Something (usually a semiconductor island) capable of confining a single electron, or a few, and in which the electrons occupy discrete energy states just as they would in an atom (quantum dots have been called "artificial atoms").

Scanning Capacitance Microscopy: A method for mapping the local capacitance of a surface.

Self-assembly: In chemical solutions, self-assembly (also called Brownian assembly) results from the random motion of molecules and the affinity of their binding sites for one another.

Smart Materials: Here, materials and products capable of relatively complex behavior due to the incorporation of nanocomputers and nanomachines. Also used for products having some ability to respond to the environment.

Technofobics: Those who have a phobia to technology, and/or to advances in technology.

Thermogravimetric Analysis (TGA): This technique provides changes in physical and chemical properties of nanoparticles / materials as a function of increasing temperature (phase transitions) such as vaporization, sublimation, absorption and desorption.

Thin film: A layer of material ranging from fractions of a nanometer (monolayer) to several micrometers (multilayer) in thickness

Thrombolysis: Thrombolysis is the process of dissolution of blood clots.

Thrombosis: Thrombosis refers to the formation of blood clots.

UV-Spectroscopy: This is a technique that relates the amount and type of radiant energy absorbed by a material to its structure, concentration and identity.

Vectors: Vectors are higher organisms that serve as carriers of pathogens.

Wet Nanotechnology: The study of biological systems that exist primarily in a water environment. The functional nanometer-scale structures of interest here are genetic material, membranes, enzymes and other cellular components. The success of this nanotechnology is amply demonstrated by the existence of living organisms whose form, function, and evolution are governed by the interactions of nanometer-scale structures.

X-Ray Diffraction (XRD): This is a rapid analytical technique primarily used for phase identification of crystalline nanoparticles.