P-3093

SYNTHESIS OF METAL SILICA NANOCOMPOSITES AND STUDIES OF ITS OPTICAL PROPERTIES

PROJECT REPORT

Submitted by

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Register No: 0820203009

in partial fulfillment for the award of the degree

Of

MASTER OF TECHNOLOGY

in

BIOTECHNOLOGY

KUMARAGURU COLLEGE OF TECHNOLOGY, COIMBATORE-06.

(An Autonomous Institution affiliated to Anna University, Coimbatore)

MAY 2010

P-3093

CERTIFICATES

ANNA UNIVERSITY: COIMBATORE

BONAFIDE CERTIFICATE

KUMARAGURU COLLEGE OF TECHNOLOGY

COIMBATORE-641 006

Department of Biotechnology

PROJECT WORK -PHASE II

MAY 2010

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(PROF. P.T. KALAICHELVAN)

DECLARATION

I affirm that the project work titled **Synthesis of Metal Silica Nanocomposites and Studies of its Optical Properties** being submitted in partial fulfilment for the award of **M.Tech** is the original work carried out by me. It has not formed the part of any other project work submitted for award of any degree or diploma, either in this or any other University.

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ACKNOWLEDGEMENT

First and foremost I thank God Almighty for his blessings for the successful completion of my project.

I wish to express my sincere thanks to **Prof. R. Rengasamy, Director,** Centre for advanced studies in Botany, University of Madras, Chennai for allowing me to pursue my project work.

I express my sincere gratitude to **P.T.Kalaichelvan, Professor,** Centre for advanced studies in Botany, University of Madras, Chennai for him valuable guidance and support throughout my project.

I am heartfully indebted to **Mr. M. Girilal, Research scholar**, Centre for advanced studies in Botany, University of Madras, Chennai for her valuable and genuine encouragement throughout the period of my project.

I wish to record my sincere gratitude to my guide **Dr.V.Stephen Rapheal,** Assistant Professor, Department of Biotechnology, for his valuable guidance and constant encouragement throughout the project.

My sincere thanks to **Dr.S.Ramachandran, Principal,** Kumaraguru College of Technology, Coimbatore, **Dr.S.Sadasivam Dean, Biotechnology,** Kumaraguru College of Technology. Coimbatore.

I wish to thank all the teaching and non-teaching members of the Department of Biotechnology, Kumaraguru College of Technology, for their help throughout my project work.

My parents remained a constant source of strength throughout my educational career to achieve my goals.

P. R. Ramya

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DEDICATED TO MY DEAR PARENTS

&

TO MY BELOVED BROTHER

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ABSTRACT

ABSTRACT

Silica-metal composite materials have attracted a great deal of interest because of their biomedical and catalysis applications. Among the noble metals, gold nanoparticles are well known for their optical property and biocompatibility, bioconjugation that play very important role in diagnostic field and as drug carrier in drug delivery system. The stability of colloidal gold is retained by the immobilization of gold nanoparticles onto the support materials. As a result, they make it easy to handle metal nanoparticles, which can decrease the cost of process and prevent the potential environmental pollution caused by the undesired spread of metal nanoparticles. Gold nanoparticles are chemically synthesized and doped in the diatom frustules made of silica skeleton. The optical properties are studied using UV-VIS spectroscopy, shows the peak at 920 nm which is near infrared region. The silica- gold nanocomposites are examined in the Scanning Electron Microscope (SEM). We have synthesised gold and silica nanocomposite using biological or biomimetic method and its optical properties was studied which will help in drug carrier and related biological applications.

Key words: Gold Nanoparticles, Diatom, SEM, Nanocomposite

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INTRODUCTION

1. INTRODUCTION

1.1. NANOTECHNOLOGY:

Nanotechnology is the creation of functional materials, devices and systems, through the understanding and control of matter at dimensions in the nanometer scale length (1-100 nm), where new functionalities and properties of matter are observed and harnessed for a broad range of applications. The transition from micro particles to nanoparticles can lead to a number of changes in physical properties. Two of the major factors in this are the increase in the ratio of surface area to volume, and the size of the particle moving into the realm where quantum effects predominate. The increase in the surface-area-to-volume ratio, which is a gradual progression, as the particle gets smaller, leads to an increasing dominance of the behavior of atoms on the surface of a particle over that of those in the interior of the particle (Paul Holister *et al.*, 2003). This affects both the properties of the particle in isolation and its interaction with other materials. High surface area is a critical factor in the performance of catalysis and structures such as electrodes, allowing improvement in performance of such technologies as fuel cells and batteries. Large surface area of nanoparticles also results in many interactions between the intermixed materials in nanocomposites, leading to special properties such as increased strength and/or increased chemical/heat resistance.

Additionally, the fact that nanoparticles have dimensions below the critical wavelength of light renders them transparent, a property which makes them very useful for applications in packaging, cosmetics and coatings. Some of the properties of nanoparticles might not be predicted simply by understanding the increasing influence of surface atoms or quantum effects. For example, it was recently shown that perfectly-formed silicon 'nanospheres', with diameters of between 40 and 100 nanometers, were not just harder than silicon but among the hardest materials known, falling between sapphire and diamond.

Nanoparticles have been used for a very long time, probably the earliest use being in glazes for early dynasty Chinese porcelain. The Lycurgus Cup made by the Romans dates to the fourth century AD. One of the very unusual features of the Cup is its colour. When viewed in reflected light, (in daylight) it appears green. When a light is shone into the cup and transmitted through the glass, it appears red. It is due to the plasmon resonance effect.

Carbon black is the most famous example of a nanoparticulate material that has been produced in quantity for decades. Roughly, 1.5 million tons of the material is produced every year. Nanotechnology is about exploiting the nanoscale nature of materials, which would exclude early use of carbon black from being given the nanotechnology label. However, new production and analysis capabilities at the nanoscale and advances in theoretical understanding of the

behavior of nanomaterials certainly mean nanotechnology can be applied to the carbon black industry.

Nanoparticles are currently made out of a very wide variety of materials. the most common of the new generation of nanoparticles being ceramics, which are best split into metal oxide ceramics, such as titanium, zinc, aluminum and iron oxides and silicate nanoparticles (silicates, or silicon oxides, are also ceramic), generally in the form of nanoscale flakes of clay. According to the most widely accepted definitions, at least one of their dimensions must be less than 100 nm, but some interesting new applications use particles of a few hundred nanometers, so this report will not be overly strict about the 100 nm limit. The nanoparticles in metal and metal oxide ceramic nanopowders tend to be roughly the same size in all three dimensions, with dimensions ranging from two or three nanometers up to a few hundred.

On the other hand, nanotechnology raises many of the same issues as with any introduction of new technology, including concerns about the toxicity and environmental impact of nanomaterials, and their potential effects on global economics, as well as speculation about various doomsday scenarios.

These concerns have led to a debate among advocacy groups and governments on whether special regulation of nanotechnology is warranted.

1.2. History of Nanotechnology:

The first use of the concepts found in 'nano-technology' was in "There's Plenty of Room at the Bottom," a talk given by physicist Richard Feynman at an American Physical Society meeting at Caltech on December 29, 1959. Feynman described a process by which the ability to manipulate individual atoms and molecules might be developed, using one set of precise tools to build and operate another proportionally smaller set, and so on down to the needed scale. In the course of this, he noted, scaling issues would arise from the changing magnitude of various physical phenomena: gravity would become less important, surface tension and van der Waals attraction would become increasingly more significant, etc. This basic idea appeared plausible, and exponential assembly enhances it with parallelism to produce a useful quantity of end products.

The term "nanotechnology" was defined by Tokyo Science University Professor Norio Taniguchi in a 1974 paper as follows: "'Nano-technology' mainly consists of the processing, separation, consolidation, and deformation of materials by one atom or by one molecule." In the 1980s the basic idea of this definition was explored in much more depth by Dr. K. Eric Drexler, who promoted the technological significance of nano-scale phenomena and devices through speeches and the books Engines of Creation: The Coming Era of Nanotechnology (1986) and Nano systems: Molecular Machinery, Manufacturing, and Computation.

Engines of Creation: The Coming Era of Nanotechnology is considered the first book on the topic of nanotechnology.

Nanotechnology and nanoscience got started in the early 1980s with two major developments: the birth of cluster science and the invention of the Scanning Tunneling Microscope (STM). This development led to the discovery of fullerenes in 1985 and carbon nanotubes a few years later. In another development, the synthesis and properties of semiconductor nanocrystals was studied; this led to a fast increasing number of metal and metal oxide nanoparticles and quantum dots. The Atomic Force Microscope (AFM) was invented six years after the STM was invented. In 2000, the United States National Nanotechnology Initiative was founded to coordinate Federal nanotechnology research and development.

1.3. Nanoparticles

In nanotechnology, a particle is defined as a small object that behaves as a whole unit in terms of its transport and properties. It is further classified according to size: in terms of diameter, fine particles cover a range between 100 and 2500 nanometers, while ultra fine particles, on the other hand, are sized between 1 and 100 nanometers. Similar to ultra fine particles, nanoparticles are sized between 1 and 100 nanometers.

"A particle having one or more dimensions of the order of 100nm or less". There is a note associated with this definition: "Novel properties that differentiate nanoparticles from the bulk material typically develop at a critical length scale of under 100nm". The "novel properties" mentioned are entirely dependent on the fact that at the nano-scale, the physics of nanoparticles mean that their properties are different from the properties of the bulk material. This makes the size of particles or the scale of its features the most important attribute of nanoparticles.

Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials. Although the size of most molecules would fit into the above outline, individual molecules are usually not referred to as nanoparticles.

1.3.1. Gold Nanoparticles:

Colloidal gold, also known as "nanogold", is a suspension (or colloid) of sub-micrometre-sized particles of gold in a fluid — usually water. The liquid is usually either an intense red colour (for particles less than 100 nm), or a dirty yellowish colour (for larger particles). Colloidal gold particles can be attached to many traditional biological probes such as antibodies, lectins, superantigens, glycans, nucleic acids, and receptors. Particles of different sizes are easily distinguishable in electron micrographs, allowing simultaneous multiple-labelling experiments (Raouf Fetni *et al* (1991).,). Colloidal gold is successfully used as a

therapy for rheumatoid arthritis in rats. An *in vitro* experiment has shown that the combination of microwave radiation and colloidal gold can destroy the beta-amyloid fibrils and plaque, which are associated with Alzheimer's disease.

In cancer research, colloidal gold can be used to target tumors and provide detection using SERS (Surface Enhanced Raman Spectroscopy) *in vivo*. To specifically target tumor cells, the pegylated gold particles are conjugated with an antibody . Gold nanorods are being investigated as photothermal agents for in-vivo applications. Gold nanorods are rod shaped gold nanoparticles whose aspect ratios tune the Surface Plasmon Resonance (SPR) band from the visible to near infrared wavelength.

1.3.2. Synthesis of Gold nanoparticles:

The gold nanoparticles exhibit unique optical, thermal, chemical and physical properties. Subsequently, strong interest is shown in the development of processes for such synthesis of nanogold particles with various shapes and sizes. Most of the current methods of preparation are based on the use of toxic chemicals, including strong reducing agents, organic solvents or surfactants. Developments of clean and eco-friendly procedures are important steps in the field of nanotechnology, as many organisms have the ability to produce such inorganic nanostructures and metal nanoparticles. The examples include the formation of iron oxides using bacteria, silica deposition in diatoms and the formation of

various metal nanoparticles (magnetic, silver, gold, palladium) using bacteria, fungi or plants. Here, we report the use of a filamentous fungus (*Trichoderma koningii*) in the synthesis of extracellular gold nanoparticles. We show that it is possible to develop a biological protocol for the shape and size controlled synthesis of gold nanoparticles, using the above mentioned fungal based biological system.

1.3.3 Optical Properties of Gold Nanoparticles

The optical properties of spherical gold nanoparticles are calculated using classical electrodynamics. The wavelength corresponding to maximum extinction shifts to longer wavelengths as the size of the nanoparticle is increased. The influence of higher-order multipoles is evident for large nanoparticles, making the spectra more complex. When the shell thickness of a core/shell particle is decreased, the Plasmon resonance shifts to longer wavelengths. This red shift is accompanied by an increase in peak intensity. A model for core/shell nanoparticles is presented to investigate surface coverage effects. This model can be used to interpret the optical properties during the growth process or to examine the effects of shell defects, uneven growth, and surface roughness. The preliminary results for low surface coverage show an increase in extinction as the number of surface particles is increased. The peak position for the array of surface particles matches

the resonant wavelength for isolated spherical nanoparticles with a radius equal to half the shell size, as expected for the uncoupled limit.

1.4. Diatoms

Diatoms are unicellular photosynthetic eukaryotes (chromophytes), within the class Bacillariophyceae in the Heterokont division, whose peculiarity amongst other microalgae is their siliceous cell wall. This wall acts as a line of defence against various types of grazers. Diatoms are thought to contribute as much as 25% of the global primary productivity (Scala and Bowler *et al.*, 2002) and their population is the largest amongst microalgae in the oceans. Amongst the eukaryotic phytoplankton, diatoms are responsible for about 40% of the marine primary productivity .The number of genera and species is in the order of 250 and 100,000, respectively. Most diatoms are unicellular, although they can exist as colonies in the shape of filaments or ribbons (e.g. *Fragillaria*), fans (e.g. *Meridion*), zigzags (e.g. *Tabellaria*), or stellate colonies (e.g. *Asterionella*). Diatoms are a widespread group and can be found in the oceans, in freshwater, in soils and on damp surfaces. Diatoms occur in all oceans from the poles to the tropics; polar and subpolar regions contain relatively few species compared with temperate biota. Although tropical regions exhibit the greatest number of species, more abundant populations are found in polar to temperate regions.

Centric diatoms are essentially planktonic microalgae which are found in all open water masses, while pennate are found most of the time in benthic forms, growing on sediments or attached to rocks or macroalgae; and some species can also be found in soil (Lee *et al.*, 1999). In their natural environment, diatoms are abundant at the beginning of spring and autumn, when nutrients are not limiting and when light intensity and day-length are optimal for diatom photosynthesis (Falciatore and Bowler *et al.*, 2002).

The cell walls are structured on a nanometer to micrometer scale, and they are reproduced precisely during each cell division cycle. Diatom cell walls are composed of inorganic and organic components. About 97% of the diatom cell wall consists of inorganic compounds, in particular almost pure hydrated silica doped with trace amounts of aluminum and iron. There is evidence from biochemical studies that the organic compounds are (glyco) proteins, and in the past few years a number of proteins associated with diatom biosilica have been purified and structurally characterized. Recently, a set of cationic polypeptides named silaffins, isolated form purified cell walls of the diatom *Cylindrotheca fusiformis* were shown to generate networks of silica nanospheres within seconds when added to a solution of silicic acid. Furthermore, high amounts of long-chain polyamines have been found in diatom cell walls. Upon addition to monosilicic

acid solution, the polyamines induce rapid precipitation of silica spheres with characteristic diameters in vitro.

1.4.1. Skeleton of Diatom

The diatom silica cell wall as a whole is called the frustule, and it is divided into two overlapping halves called thecae: the upper being the epitheca and lower being the hypotheca. The general structure of a diatom frustule is similar to a Petri dish consisting of valves, which are the distinctive species-specific structures capping the top and bottom, and girdle bands, which are a series of overlapping siliceous strips that surround the cell to form the sides, and also provide overlap between the two thecae. There are two general morphological types of diatoms based on their valve structures: the centrics, which have rotational symmetry, and the pennates, which have bilateral symmetry (Round *et al.*, 1990). Diatom silica is made from the soluble precursor form of silicic acid, which is transported into the cell (Hildebrand *et al.*, 1997 &1998) and into a specialized membrane bound compartment called the silica deposition vesicle (Crawford *et al.*, 2001) or SDV, where it is polymerized to form the mineral. Once the mineral structure of the valve or girdle band is completed, it is moved in its entirety across the plasma membrane (exocytosed), where it becomes an exoskeleton surrounding the cell. There are three scales of silica structure formation in diatoms, the micro-, meso-, and nanoscale (Hildebrand *et al.*, 2006). Determinants involved in the formation of

micro- and nano-scale structures are at least partially characterized, but we understand little about what generates the mesoscale. The microscale is the overall outline structure of the valves and girdle bands, which is determined by shaping of the SDV through active and passive molding. A major aspect of this shaping involves components of the cytoskeleton – actin and microtubules, which apparently dynamically define and expand the SDV (van deMeene *et al.*, 2002). Much more remains to be determined about the interaction of the cytoskeleton and SDV, how the overall SDV shape is defined by the cytoskeleton, and how SDV membrane components interact with the cytoskeleton. Structures on the nanoscale are formed as a result of the initial polymerization of silica and manifest as the silica "texture" seen in high-resolution images. Characterization of organic components tightly associated with diatom silica suggests that major determinants of nanoscale structure are long chain polyamines (LCPAs), which catalyze silica polymerization (Kroger *et al.*, 2000) and silaffins, which are polypeptides proposed to play a regulatory role in silica polymerization by organizing LCPAs via electrostatic interactions (Poulsen *et al.*, 2004). In vitro, LCPAs and silaffins isolated from diatoms precipitate silica to form a variety of morphologies, but these lack the higher order structure seen in a diatom frustule. In diatoms, higher order is seen on the mesoscale, in which substructures consisting of organized patterns of nanoscale polymerization products are formed within the confines of

the microscale of the SDV. Little is known about mesoscale organizational components, but as most of the species-specific differences in diatom silica structure occur at the mesoscale, further investigations will be important to understand how these components contribute to generating species-specific differences.

1.4.2. Mechanical Properties of Diatoms

Defense against predation or damage by means of a mechanically sound armor is a common mechanism in biology, and the diatom silica frustule is certainly a masterpiece of such defense. In the environment, diatoms are exposed to many mechanical challenges, such as abrasive particles and solid sediments, but also to changes in osmolarity, which could lead to swelling or bursting of unprotected cells. Together, frustule valves and girdle bands form pill boxlike protection, completely surrounding the plasma membrane. Mechanical protection using diatom biosilica requires lower material investment and less energy in comparison with other biominerals (calcite) (Wetherbee *et al.*, 2004).

1.5. Advantages and Limitations of SEM, TEM, and AFM to Image Diatom Biominerals

Characterization of diatoms at high resolution has relied upon SEM or TEM imaging. SEM has the advantage of high resolution (depending on the instrument), the ability to image large changes in height and large areas. Its

limitations are that coatings are sometimes required, which could mask or distort some nanoscale features, and its inability to obtain small-scale height information. TEM has high resolution, but the electron transparency of many materials (including biosilica) can make it difficult to distinguish between different layers, and surface imaging is only possible with a thin section containing a surface slice or in cross-section. AFM has the highest resolution of the three techniques and can provide nanoscale height information and the most detailed surface topography, without the need for coatings. Its limitations are that it can be difficult to image large changes in height due to the limits posed by the movement of the Z piezo scanning element that in most AFM's is limited to about 10 μm. Another limitation for imaging the larger diatom is the XY scan range that is limited to about 100 μm. The radius of curvature of the AFM cantilever tip also causes a broadening of structure in the lateral dimensions, and care must be taken when interpreting structure in this plane (Thundat *et al.*, 1992).

OBJECTIVES

2. OBJECTIVES

- To isolate different types of diatom species from the marine water of various areas.
- To synthesize metal silica nano composites from the isolated diatoms.
- To analyze the optical properties of metal silica nano composites.

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

Nanotechnology creates structures that have excellent properties by controlling atoms and molecules, functional materials, devices and systems on the nanometer scale by involving precise placement of individual atoms (around 0.1-100 nm, one nanometer is one millionth of a meter). Nanotechnology brings new functions and properties to develop new products and applications in the industrial fields such as chemistry, medical technology, automobile, food industry, pharmacy, textile industry, environmental industry and biotechnology where nanoscale is so important. Nano technology is an interdisciplinary science branch, which takes role in the material science, mechanics, electronics, optics, medicine, plastics, energy, aerospace, textiles, optical coatings, photovoltaics, antibacterial agents, physics and biology. Although the meaning and the structure of the nanotechnology are very complex, it takes great part in our daily life because lots of the products that are used in our everyday life can be developed by nanotechnology. (Marcato *et al.*, 2005).

3.1. Synthesis of Nanoparticles

Metals such as gold, silver can be synthesized both by chemical method and biological method.

3.1.1. Chemical Synthesis

Silver nanoparticles were prepared by chemical reduction method. Silver nitrate was taken as the metal precursor and hydrazine hydrate as a reducing agent. The formation of the silver nanoparticles was monitored using UV-Vis absorption spectroscopy. The UV-Vis spectroscopy revealed the formation of silver nanopartícles by exhibing the typical surface plasmon absorption maxima at 418-420 nm from the UV–Vis spectrum (Maribel *et al.*, 2009).

Colloidal gold nanoparticles are used as building blocks for fabrication of anisotropic and multicomponent nanoparticles (e.g., nanoshells, semiconductor nanocrystals, and gold nanorods). The solution synthetic methods developed in this paper demonstrated that gold NPs can be used as a multifunctional nanoplatform to fabricate metallic nanoshells, anisotropic semiconductor nanocrystals, and gold NRs at mild conditions. The ability to easily fabricate these high quality nanomaterials in high yield will be valuable in applications such as bioimaging and bioassay technologies, light-emitting diodes, and photovoltaics. These studies provide a new direction in developing facile syntheses of different classes of NPs with unique tunable optical properties and thereby making available new building blocks for bionanotechnology. The tunable optical properties of these nanoparticles are well suited for various biomedical and biophotonic applications (Ken-Tye Yong *et al.*, 2009).

3.1.2. Biological Synthesis

Microorganisms such as bacteria, yeasts, algae, fungi and actinomycetes are used in the biosynthesis of metal nanoparticles. *Verticillium* sp. and *Fusarium oxysporum*, when exposed to aqueous gold and silver ions, reduced the metal ions fairly rapidly to nanosize. In the case of *Verticillium* sp., reduction of the metal ions occurred intracellularly leading to the formation of gold[53] and silver[54] nanoparticles in the size range 2–20 nm. *F.oxysporum* behaved considerably differently, the reduction of the metal ions occurring extracellularly resulting in the rapid formation of highly stable gold[55] and silver[56] nanoparticles of 2–50 nm dimensions. *Thermomonospora* sp. when exposed to gold ions reduced the metal ions extracellularly, yielding gold nanoparticles with a much improved polydispersity . One or more of these proteins may be enzymes that reduce chloroaurate ions and cap the gold nanoparticles formed by the reduction process. It is also possible that the capping and stabilization of the gold nanoparticles is effected by a different protein Extra cellular secretion of enzymes offers the advantage of obtaining large quantities in a relatively pure state, free from other cellular proteins associated with the organism and can be easily The use of fungi and actinomycetes as sources of enzymes that can catalyse specific reactions leading to inorganic nanoparticles is a new and rational biosynthesis strategy. The use of specific enzymes secreted by organisms such as fungi in the synthesis of

nanoparticles is exciting for the following reasons. The process can be extended to the synthesis of nanoparticles of different chemical compositions and indeed, different shapes and sizes by suitable identification of enzymes secreted by the fungi. Understanding the surface chemistry of the biogenic Nanoparticles (i.e. nature of capping surfactants/peptides/proteins) would be equally important. This would then lead to the possibility of genetically engineering microbes to overexpress specific reducing molecules and capping agents and thereby, control the size and shape of the biogenic Nanoparticles (Murali Sastry *et al* ., 2003).

Beveridge and Murray (1980) have demonstrated that gold NPs readily precipitate in bacterial cells following incubation of the cells with Au^3 ions under ambient temperature and pressure. Organic phosphate compounds play a role in the in vitro development of octahedral Au, possibly as bacteria–Au complexing agents. In the presence of S. algae and hydrogen gas, the Au ions are completely reduced and 10–20 nm gold NPs are formed (Konishi *et al*.,2004).

Kinetic measurements of enzyme activity for gold NP synthesis will then be undertaken at the optimal pH and temperature, as determine in separate experiments. The shape controlled synthesis of gold NPs by purified enzyme(s) will be also performed using varying reaction conditions, such as the concentration of gold ions, the pH of the solution, and the incubation period. All these conditions control the crystal growth kinetics and final NP morphology. Since the enzyme(s)

secreted by R. oryzae act both as reducing and capping agent, the adsorption of such enzyme(s) on the growing crystals and their reducing activity changes with the various conditions, thereby resulting in different crystal shapes

(Sujoy *et al* ., 2010)

Extracellular biosynthesis of gold nanoparticles using *Sargassum wightii* have achieved rapid formation of gold nanoparticles in a short duration. The UV–vis spectrum of the aqueous medium containing gold ion showed peak at 527 nm corresponding to the plasmon absorbance of gold nanoparicles. Transmission electron microcopy (TEM) showed formation of well-dispersed gold nanoparticles in the range of 8–12 nm. X-ray diffraction (XRD) spectrum of the gold nanoparticles exhibited Bragg reflections corresponding to gold nanoparticles. (Singaravelu *et al*., 2007).

3.2. Synthesis of Metal -Silica Nanocomposites

The one-step synthesis of a silica-gold nanocomposite by simultaneous hydrolysis and reduction of gold chloride. The aminophenyl group was used as a reducing agent, and the trimethoxy silane group acts a precursor for the formation of silica. The porous gold nanoparticles were formed by etching out the silica-gold nanocomposites by hydrofluoric acid. The electron diffraction of porous gold nanoparticles showed that the particle are polycrystalline with FCC structure. The silica-gold nanocomposite exhibited nonlinear current-voltage behavior, and the

porous gold nanoparticles displayed linear current-voltage behavior. (Ashavani Kumar *et al.*, 2006)

Gold colloids have been homogeneously coated with silica using the silane coupling agent (3-aminopropyl)- trimethoxysilane as a primer to render the gold surface vitreophilic. After the formation of a thin silica layer in aqueous solution, the particles can be transferred into ethanol for further growth using the Sto¨ber method. The thickness of the silica layer can be completely controlled, and (after surface modification) the particles can be transferred into practically any solvent. Varying the silica shell thickness and the refractive index of the solvent allows control over the optical properties of the dispersions. The optical spectra of the coated particles are in good agreement with calculations using Mie's theory for core-shell particles (Luis *et al.*, 1996)

Au-silica heterogeneous nanocomposite particles were prepared by novel preparation strategy involving alcohol-reduction method using 3- minopropyltrimethoxysilane (APTMS) as a binder between silica surface and Au nanoparticles, and using PVP as a stabilizer for Au nanoparticles. The evolution of morphology of composite particles was investigated with increasing reaction time at different Au precursor concentrations of 100 ppm and 250 ppm using UV–vis spectrophotometer and TEM. It is shown that the size of immobilized Au particles on silica surface can be controlled with the variation of

microparticles from the intestinal lumen to the bloodstream led to distribution of substances in the body.

The mechanism of diatom cell division is studied by means of ^{29}Si isotope tracing (Audinot *et al.*, 2006). During cell division, each cell produces two daughter cells, each of them keeping one of the two valves of the mother cell and producing a new valve by absorbing the silicon present in the environment. The NanoSIMS 50 allows ion imaging to be performed on diatoms in order to determine the site of fixation of silicon. Different types of diatoms have been transferred in a culture medium enriched with ^{29}Si and after several days, the distribution of the different isotopes of silicon has been determined by NanoSIMS 50 imaging ^{29}Si enriched valve is a newly formed valve and that to construct this new valve, the silicon of the ^{29}Si enriched culture medium has been absorbed during the multiplication. The isotopic ratio $^{29}Si/^{28}Si$ has been determined.

Diatoms generate their cell walls by silica biomineralization. The cell walls are composed of silica and organic macromolecules and show a complex microscopic structure. Analysis of this structure by different atomic force microscopy (AFM) techniques revealed an unexpected nanostructured granular surface. Silaffins, proteins that are posttranslationally modified with long-chain polyamines and oligo-*N*- methylpropyleneamines were identified as the main organic constituents of diatom biosilica. Silaffins as well as free propylene amines

The size-dependent physicochemical and optical properties of silica nanoparticles have been studied. Significant increase in the specific surface area (SSA), concentration of silanol groups (ıOH) and apparent density (*D*a) were observed as the particle size reduced from 130 to 7 nm. The decrease in the silanol number (ˌOH) and Si–O–Si bond angle in smaller particle size suggest that the silica structure, especially the surface has been significantly altered at nanoscale. This finding is supported by the presence of defect sites such as E-centers and oxygen deficient centers (OCD). The stability of E_ centers (UV–vis analysis) increase linearly with the increase in particle size. The increase in the intensity of blue and green bands (PL analysis) with the decrease in the particle size are attributed to the higher silanol concentration and increased in the number of self-trapped exciton (STE)/OCD, respectively. The green bandwas blue-shifted with the decrease in the particle size. Overall, the silica nanoparticles have shown distinctive properties relative to the bulk silica. (Rahman *et al* ., 2009)

3.4. DIATOMS

The procedure adopted in the preparation of the artificial medium, and in the culture of the diatoms, was, with a few modifications, essentially that described by Allen and Nelson (1910).

Hussain *et al.* (2001) showed cell capture of microparticulate substances by enterocytes, and their transport between cells. In some cases, the passage of

reaction rate via adjusting Au precursor concentration and reaction time, but the high concentration of Au precursor hinders the immobilization of well-defined Au nanoparticles due to the slow reduction rate of Au precursor. On the basis of experimental results, the role of APTMS and PVP on the formation of composite particles and the effect of Au precursor concentration on the morphological evolution of composite particles are briefly discussed.(Tae-Hyun *et al.*, 2008)

Gold nanoparticles with an average size of _5 nm were deposited on the surface of preformed silica submicrospheres with the aid of power ultrasound. The sonochemical reduction was carried out by ultrasonic irradiation in an argon atmosphere at room temperature. Ultrasonic irradiation of a slurry of silica submicrospheres, chloroauric acid (HAuCl4), and ammonia in an aqueous medium for 45 min yielded a gold-silica nanocomposite. By controlling reaction conditions, we could achieve the deposition of metallic gold on the surface of the silica spheres. A unique crystallization process of the silica particles is observed. The crystallization process is assisted by the gold nanoparticles yielding the cristobalite phase of silica at a relatively low temperature. The resulting gold-deposited silica submicrosphere samples were characterized with XRD, EDAX, TEM, TGA, DSC, HR-SEM, and FT-IR, photoacoustic, and UV-visible spectroscopy.(Pol *et al.*, 2003)

The gold nanostructures are formed by means of thermal evaporation of gold onto porous frustules. Nanostructured gold films were obtained upon release from the diatom templates and were characterized using scanning electron microscopy (SEM), atomic force microscopy (AFM) and UV-Vis spectrophotometry. Three centric diatom species, Coscinodiscus sp., Thalassiosira eccentrica and one unidentified species cultured in our laboratory, were used as templates. The prepared gold replicas come in a variety of forms and shapes including arrays of nanoscale pillars, dots and more complex three-dimensional structures, depending on which porous surface of the diatom was used for replication. In all cases, gold nanostructures closely follow the organization and distribution of pores of the frustule template. Spectrophotometric characterisation shows that the templated nanostructured gold films exhibit localized surface plasmon resonance (LSPR) effects. (Dusan 2006)

3.3. Optical Properties of Metal Nanoparticles

Metal nanoparticles, especially gold and silver exhibit many unique properties such as surface plasmon resonance, surface enhanced Raman scattering, non-linear optical properties, and quantized charging effect (Daniel & Astruc 2004).

core-shell (silver core-silica shell) nanoparticles with various shell thicknesses featuring a variety of fluorophores, to show the versatility of the core-shell architecture, and have demonstrated their applicability for two platform technologies, metal-enhanced fluorescence (MEF) and single nanoparticle sensing. (Kadir *et al.*, 2007).

The optical properties of spherical gold nanoparticles are calculated using classical electrodynamics. The wavelength corresponding to maximum extinction shifts to longer wavelengths as the size of the nanoparticle is increased. The influence of higher-order multipoles is evident for large nanoparticles, making the spectra more complex. When the shell thickness of a core/shell particle is decreased, the plasmon resonance shifts to longer wavelengths. This red shift is accompanied by an increase in peak intensity. A model for core/shell nanoparticles is presented to investigate surface coverage effects. This model can be used to interpret the optical properties during the growth process or to examine the effects of shell defects, uneven growth, and surface roughness. The preliminary results for low surface coverage show an increase in extinction as the number of surface particles is increased. The peak position for the array of surface particles matches the resonant wavelength for an isolated spherical nanoparticle with a radius equal to half the shell size, as expected for the uncoupled limit (Cleveland Eugene *et al* ., 2005)

of different chain lengths induce rapid precipitation of nanosized particles from silicic acid solutions in vitro. In a biomimetic approach, we reacted aqueous silicic acid solution with tripropylenetetramine in CHCl3 in a biphasic system. As a result, thin nanostructured silica layers that show a granular nanostructure very similar to that of the diatom cell walls were obtained. This finding may serve as a good model to study the mechanisms that lead to the nanostructure of the diatom cell walls (Frank Noll et al., 2002).

3.5. Understanding Diatoms using Atomic Force Microscope (AFM)

AFM proves ideal for imaging even thin Girdle band structures, and a wealth of details on micro-, meso-, and nanoscale features are apparent. AFM imaging reveals similar construction features in the girdle bands as in the valves—linear features perhaps relating to an underlying template on the proximal surfaces and less ordered silica particles on the distal surfaces. This suggests that template association with one face of the silica-depositing vesicle (SDV) may be a general feature of formation of different structures in diatoms. Continuing application of AFM to study girdle bands is likely to be fruitful in understanding the mechanisms of formation and similarities and differences between girdle band and valve formation. AFM not only provides information on silica structures but also gives insight into features of possible underlying organic templates that may be involved in structure formation. One general theme revealed in this study (Mark Hildebrand

et al., 2008) is the prevalence of linear mesoscale templates, even in diverse complex-curved valves, girdle bands, and setae microscale structures. This suggests the likelihood of a class of linearly assembling SDV-associated proteins involved in organizing silica polymerization determinants.

3.6. APPLICATIONS

Gold nanoparticles (AuNPs) provide non-toxic carriers for drug and gene delivery applications. With these systems, the gold core imparts stability to the assembly, while the monolayer allows tuning of surface properties such as charge and hydrophobicity. An additional attractive feature of AuNPs is their interaction with thiols, providing an effective and selective means of controlled intracellular release (Partha Ghosh *et al.*, 2008)

Novel multifunctional magnetic gold nanocomposites (MGNCs) were synthesized for synchronous cancer therapy and diagnosis via magnetic resonance imaging (MRI). The MGNCs consist of magnetic kernels (aggregates of ultra-sensitive MnFe2O4 magnetic nanocrystals wrapped in polymer) as effective MR contrast agents and silica–gold nanocomposites as hyperthermal therapeutic agents. A therapeutic antibody, Erbitux (ERB), was conjugated for specific tumor cell targeting both to localize the near-IR laser beam and to image their events through MRI. ERB-conjugated MGNCs selectively recognize the target cancer

cell lines. Fluorescence images and MRI analysis show that the MGNCs are effectively taken up by the cells. ERB-conjugated MGNCs have an excellent synchronous therapeutic efficacy as a result of the therapeutic antibody and near-IR laser-induced surface plasmon resonance. Consequently, MGNCs clearly demonstrate selective imaging and treatment of human epithelial cancer simultaneously.(Jaewon Lee *et al.*, 2008)

Gold nanoparticles (GNPs) are used in human hepatocellular and pancreatic cancer cells to determine: 1) absence of intrinsic cytotoxicity of the GNPs and 2) external radiofrequency (RF) field-induced heating of intracellular GNPs to produce thermal destruction of malignant cells. GNPs (5 nm diameter) were added to 2 human cancer cell lines (Panc-1, Hep3B). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and propidium iodide-fluorescence associated cell sorting (PI-FACS) assessed cell proliferation and GNP-related cytotoxicity. Other GNP-treated cells were exposed to a 13.56 MHz RF field for 1, 2, or 5 minutes, and then incubated for 24 hours. PI-FACS measured RF-induced cytotoxicity. (Christopher *et al.*, 2008)

Mesoporous silica nanoparticles functionalized by surface hyperbranching polymerization of poly(ethylene imine), PEI, were further modified by introducing both fluorescent and targeting moieties, with the aim of specifically targeting cancer cells. Owing to the high abundance of folate receptors in many cancer cells

as compared to normal cells, folic acid was used as the targeting ligand. The internalization of the particles in cell lines expressing different levels of folate receptors was studied. Flow cytometry was used to quantify the mean number of nanoparticles internalized per cell. Five times more particles were internalized by cancer cells expressing folate receptors as compared to the normal cells expressing low levels of the receptor. Not only the number of nanoparticles internalized per cell, but also the fraction of cells that had internalized nanoparticles was higher. The total number of particles internalized by the cancer cells was, therefore, about an order of magnitude higher than the total number of particles internalized by normal cells, a difference high enough to be of significant biological importance. In addition, the biospecifically tagged hybrid PEI-silica particles were shown to be noncytotoxic and able to specifically target folate receptor-expressing cancer cells also under coculture conditions (Jessica *et al.*, 2009).

Biosilicified nanostructured microshells from the marine diatom Coscinodiscus wailesii have been properly functionalised to bind a molecular probe which specifically recognises a target analyte. The chemical modification process has been characterised by Fourier transformed infrared spectroscopy. Fluorescence measurements demonstrate that the antibodies we used, even if linked to the amorphous silica surface of C. wailesii microshells, still efficiently recognise their antigens. These low cost and largely available natural materials can

be thus used as transducers elements for optical biosensors or as targeting microcapsules for drug delivery (De Stefano *et al.*, 2008)

3.7. Bionanotechnology

A major goal for bionanotechnology is the development of microscale total analysis systems (μTAS) or lab-on-a-chip (LOC). The frustule architecture of the giant diatom *Coscinodiscus* also dramatically increases the surface area (Helen *et al.*, 2008). An anti-IgY (IgY: immunoglobulin Y) antibody raised in rabbit was bound to the silica surface of the diatom using silane chemistry. The surface of the diatom was silanized and then treated with a heterobifunctional cross linker, and an antibody against tubulin was tethered to the diatom frustule via carbohydrate groups. The silica walls can be used for the attachment of active biomolecules, such as antibodies, using either primary amine groups or the carbohydrate moiety. These modified structures can, therefore, be used for antibody arrays or for use in techniques such as immunoprecipitation. Furthermore, diatoms produce these silica structures without the need for industrial chemical processes, providing an exceptionally cheap material limited only by the need for basic nutrients and light.

In nanoengineering, progress has been achieved in converting diatom biosilica into a range of industrially relevant materials including zeolitic, ceramic, and metallic materials under preservation of the nanostructured membrane architecture. The patterns of 3D diatom frustules have been further converted into

2D nanostructures by replica-molding approaches. Experimentation with the diatom culture medium has provided opportunities for control over biosilica membrane pore size, pore morphology, the frustule's degree of silicification, and the incorporation of chemicals or biologicals. In optics, the luminescence of diatom silica provides new opportunities for the design of complex nanoscale microlensing and waveguiding systems, as well as providing unexploited lessons for efficient harvesting and control of light using nanoscale structures. The ability of diatoms to engineer strong and robust adhesives that are stable in wet environments has inspired new adhesive materials. Diatoms have also developed chemistries to address tribological problems such as frictional wear and tear at the micro- and nanoscale, by producing effective lubricating agents and devising self-healing adhesives. Optical and electrochemical transducers for the detection of gases and organic vapors have been realized, exploiting the luminescence properties of diatom silica and the semiconducting Properties of chemically altered diatom frustules, respectively. Advances in interfacing bioactive molecules, including antibodies and DNA, have enabled the use of diatoms as miniaturized immuno and DNA sensors compatible with LOC platforms (Wetherbee *et al.*, 2004).

Biosilicified nanostructured microshells from the marine diatom Coscinodiscus wailesii have been properly functionalized to bind a molecular

probe, which specifically recognizes a target analyte (De Stefano *et al.*, 2008). The murine monoclonal antibody (MoAb) UN1, selected for the specific reactivity with human thymocytes as compared to peripheral blood cells has been used as molecular bioprobe. The biochemically-modified surface of diatoms frustules have been tested in covalently immobilize with rhodamine labelled antibodies. The frustule-MoAb system is very stable. Chemically modified the surface of diatom frustules and proved by fluorescence macroscopy measurements that the antibodies linked covalently on the diatoms surface still recognise specifically their antigens This could be the first step in realise a diatom-based luminescent biosensor since these natural occurring microshells show a well known photoluminescence when laser irradiated. We would like to underline that the intricate structure of diatoms frustules offer a lager surface area respect to a planar glass slide and also to silica microbeads so that, beside the low cost material, better performances in biotechnological applications could be foreseen.

3.8. Health Risks of Nanotech (Peter HM Hoet *et al.*, 2004):

Lung:

The pathogenic effects of inhaled solid material depend primarily on achieving a sufficient lung burden .The smaller the particulates the deeper they can travel into the lung, particles smaller than 2.5 micron will even reach the

alveoli. Ultra fine particles (nanoparticles with an aerodynamic diameter of less than 100 nm) are deposited mainly in the alveolar region.

Intestinal tract:

Particles (0.1–1.0 micron) are associated with the Crohn's disease and indicated as potent adjuvant in model antigen-mediated immune responses.

Skin:

TiO2 particles are often used in sunscreens to absorb UV light and therefore to protect skin against sunburn or genetic damage. That micrometer-sized particles of TiO2 get through the human stratum corneum and even into some hair follicles – including their deeper parts penetration of the skin barrier is size dependent, nano-sized particles are more likely to enter more deeply into the skin than larger ones.

Body distribution and systemic effects of Particulates:

The body distribution of particles is strongly dependent on their surface characteristics. For example, coating poly (methyl methacrylate) nanoparticle with different types and concentrations of surfactants significantly changes their body distribution.

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1 Materials Required

4.1.1. Preparation of F/2 Medium (Guillard and Ryther *et al.*, 1962)

To 950 ml of filtered seawater, add following compounds listed below;

Quantity	Compound	Stock solution in distilled water	Molar concentration in final medium
1 ml	Na_2NO_3	75g/L	$8.83 * 10^{-4}$ M
1 ml	$Na_2PO_4 . H_2O$	5 g/L	$3.63 * 10^{-5}$ M
1 ml	f/2 trace metal solution	–	–
0.5 ml	f/2 vitamin solution	–	–

Table no 4.1: Preparation of F/2 Medium

Make the final volume to 1 litre with filtered seawater and autoclave at 121^0 C, 15 psi.

4.1.2. Preparation of f/2 Trace Metal Solution

To 950 ml of distilled water, add the following compounds listed below;

Quantity	Compound	Stock solution in distilled water	Molar concentration in final medium
3.15 g	$FeCl_3 . 6H_2O$	_	$1* 10^{-5}$ M
4.36 g	$Na_2EDTA . 2H_2O$	_	$1* 10^{-5}$ M
1 ml	$CuSO_4 . 5H_2O$	9.8 g/L	$4* 10^{-8}$ M
1 ml	$Na_2MoO_4 . 2H_2O$	6.3 g/L	$3* 10^{-8}$ M
1 ml	$ZnSO_4 . 7H_2O$	22.0 g/L	$8* 10^{-8}$ M
1 ml	$CoCl_2 . 6H_2O$	10.0 g/L	$5* 10^{-8}$ M
1 ml	$MnCl_2 . 6H_2O$	180.0 g/L	$9* 10^{-7}$M

Table no 4.2: Preparation of f/2 Trace Metal Solution

Make the final volume to 1 litre with distilled water and autoclave at 121^0 C, 15 psi.

4.1.3. Preparation of f/2 Vitamin Solution

To 950 ml of distilled water, add the following compounds listed below;

Quantity	Compound	Stock solution in distilled water	Molar concentration in final medium
1 ml	Vitamin B_{12}	1 g/L	$1* 10^{-10}$ M
10 ml	Biotin	0.1 g/L	$2 * 10^{-9}$ M
200 mg	Thiamine . HCl	_	$3 * 10^{-7}$ M

Table no 4.3: Preparation of f/2 Vitamin Solution

Make final volume upto 1L of distilled water. Autoclave at 121^0 C, 15 psi and store in refrigerator.

Note:

Preparation of Vitamin B_{12}:

Allow 11% water for crystallization (for each 1.0 mg of Vitamin B_{12} add 0.89 ml of distilled water).

Preparation of Thiamine. HCl:

Allow 4% water for crystallization (for each 1.0 mg of Vitamin B_{12} add 9.6 ml of distilled water).

4.2 Methodology (Richard M. Knuckey *et al.*, 2002):

4.2.1. Collection of samples

Water samples are collected from two places namely Injambakkam, Kovalam and Pondicherry beaches. The samples collected are filtered using 5-µm nylon screen and washed in the seawater such that diatoms in the screen are detached. This is the required sample.

4.2.2. Isolation of diatom

F/2 medium (Table no. 4.1) is autoclaved at 121^0 C, 15 psi. Pour 20ml of the f/2 medium into sterilized petriplates. The collected samples were serial diluted in distilled water to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} dilutions. 100 µl of dilutions, $10^{-5}$and 10^{-6} are added to 100 ml of f/2 liquid broth medium.

4.2.3. Culture conditions

Inoculated plates were maintained at 15±2 °C on a 12:12 h light/dark cycle with cool white fluorescent lighting of 100 µmol photons PAR .m^{-2} s^{-1} intensity.

4.2.4. Isolation of Single Diatom Species

20 µl of diatom broth culture is added on a clean glass slide and viewed under light microscope. Diatoms of different shapes and sizes are seen. Using the micropipette the single diatom species is picked from the mixed culture. The single diatom is inoculated in 100 ml of autoclaved f/2 broth medium. The same culture conditions mentioned above is followed. After 20 days, pure culture of single species of diatom is obtained.

4.2.5. Subculture of diatoms

Subculture is done by adding 100 µl of culture to 100 ml of f/2 broth medium autoclaved at 121^0 C, 15 psi.

4.2.6. Acid Treatment

The broth cultures are centrifuged at 10,000 rpm for 15 minutes. Add 10 ml of concentrated sulphuric acid and hydrochloric acid in 1:1 ratio to the pellet. The diatoms are maintained in the acid treatment for 72 hours. The pellets are washed in glass-distilled water by centrifuging at 10,000 rpm for 15 minutes. The centrifugation repeated several times until chloroplast is removed from the diatom.

4.2.7. Identification of Diatoms

The genus of isolated diatoms is found by microscopic identification of diatoms from School of Algal Science, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai.

4.2.8. Synthesis of Silica- Gold Nanocomposites

10ml solution of 2 M HAuCl4 is added to pellet of diatom culture devoid of chloroplast and incubated for 15 minutes. 20 µL of Hydrazine Hydrate is added to reduce the HAuCl4 to elemental Au. The gold nanoparticle synthesized is incubated with the diatom for 24 hours to allow perfect binding of gold nanoparticles to the pores of silica frustules.

4.2.9. Characterization of Silica-Gold Nanocomposites

The optical properties of the gold- silica nanoparticles are studied by the UV-VIS Spectrophotometer and Scanning Electron Microscope (SEM).

UV-VIS Spectrophotometer:

For UV - Vis the scanning mode is taken in the range of 400nm to 1000 nm. Normal glass distilled (GD) water is used as the blank for the experiment.

Scanning Electron Microscope (SEM):

Scanning Electron Microscope (SEM), Hitachi is used to observe the silica skeleton of the diatom. The pellet is kept in minimum amount of 80% ethanol and took for imaging. Ethanol is used for easy drying of sample.

RESULTS AND DISCUSSION

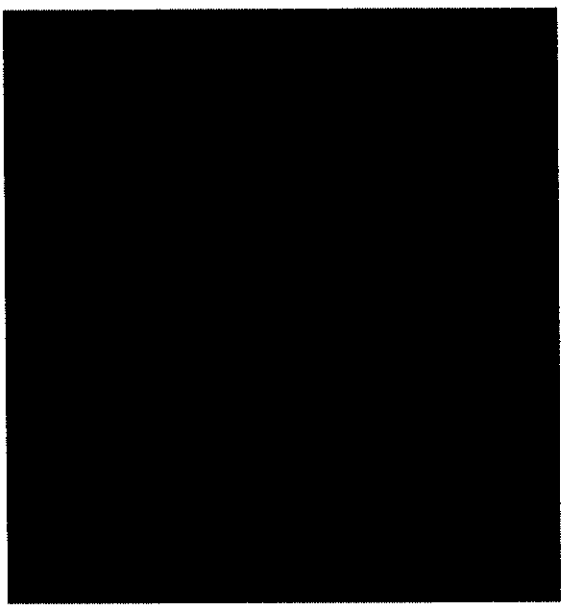
5. RESULTS AND DISCUSSION

Sea water samples were collected from different parts of Chennai and Pondicherry and grown in specialized F/2 medium. From this three individual cultures were isolated and maintained in the medium (Fig no. 5.1). This is kept for complete removal of chlorophyll and other pigments by sequential acid washing. The marine diatom cultures were identified with the morphological observation with the help of optical microscope and Scanning Electron Microscopical studies as *Navicula* sp., *Pleurosigma* sp., *Amphora sp* (Fig no. 5.2) from School of Algal Science, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai.

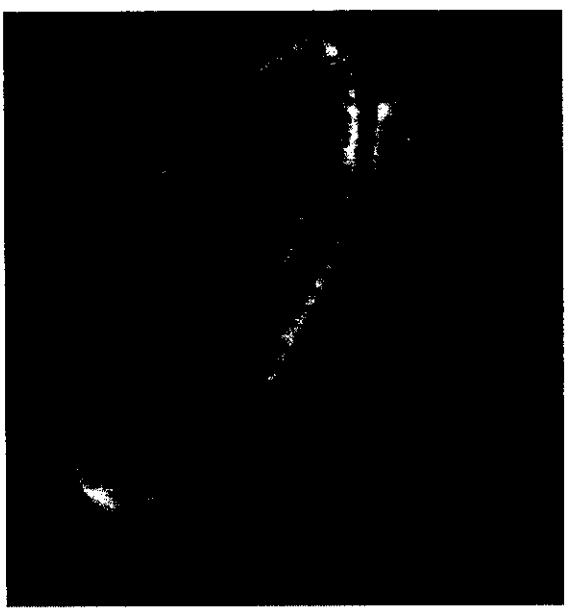
Single pure culture was isolated from this and grown in F/2 medium and was identified by using the well known manuals of Desikachary (1986, 1987, 1988 and 1989) according to the morphology, arrangements of raphe and striae. The isolated organisms were identified as *Navicula* sp., *Pleurosigma* sp. and *Amphora sp* (Fig no. 5.2).

Fig no. 5.1: Diatom Culture Maintained in the Culture Room

Amphora sp



Navicula sp.



Pleurosigma sp.

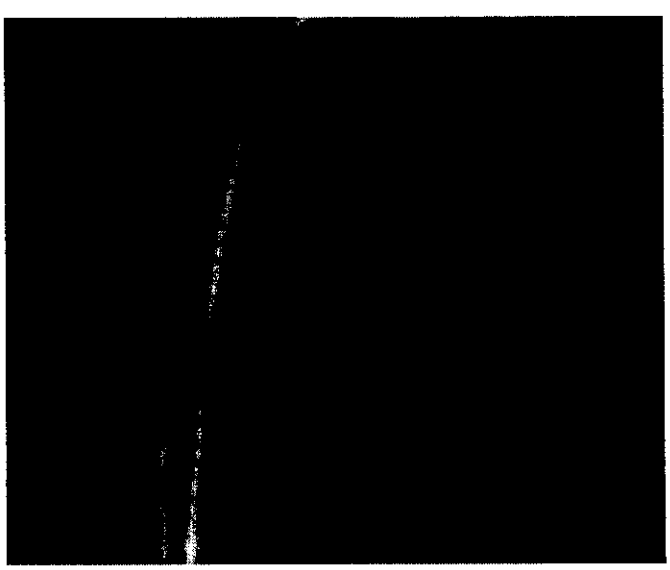


Fig no. 5. 2: Isolated Marine Diatoms

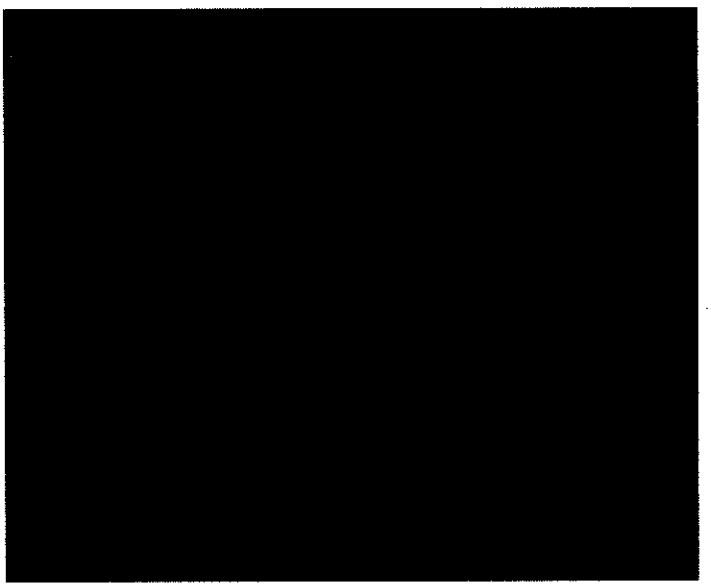


Fig no. 5. 3: *Amphora sp* Control

Fig no. 5. 4: *Amphora* sp – Au Treated

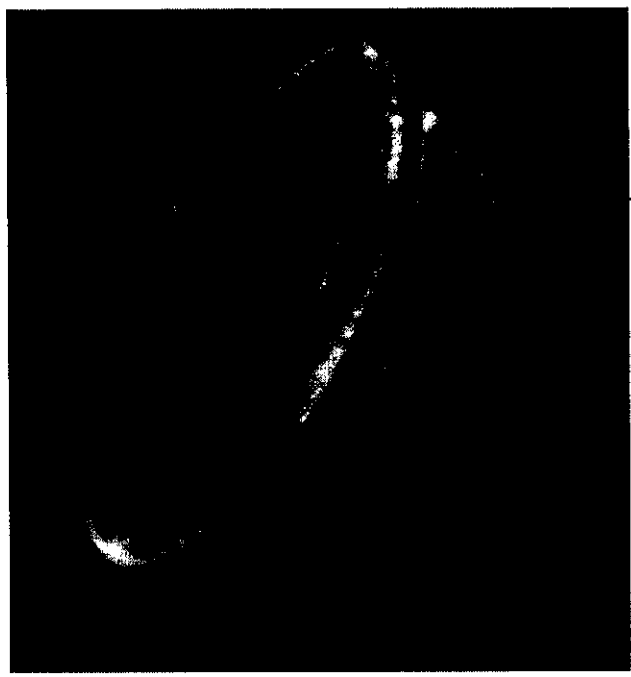


Fig no. 5. 5 : *Navicula* sp. Control

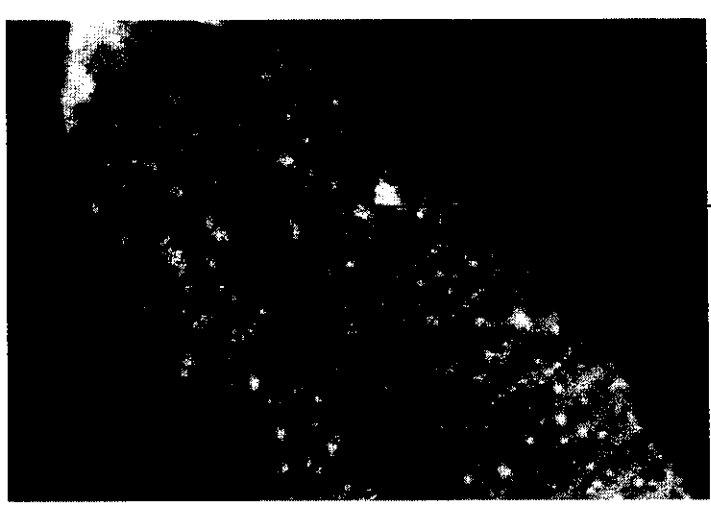


Fig no. 5. 6: *Navicula* sp. – Au Treated

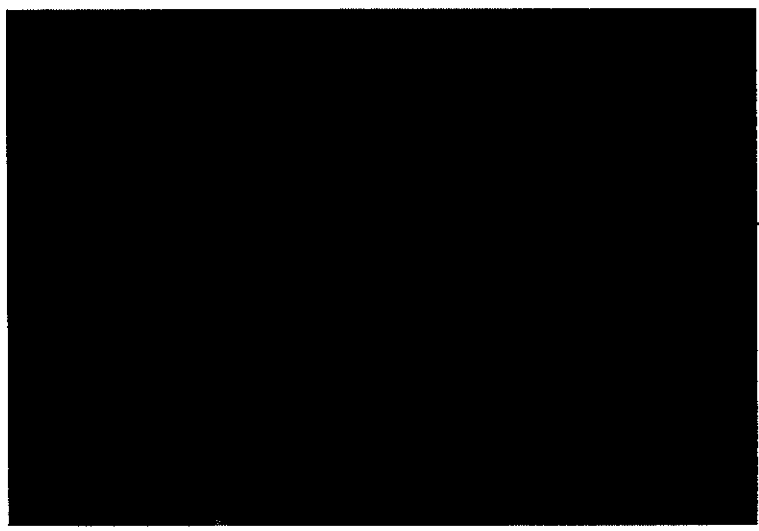


Fig no. 5. 7: *Pleurosigma* sp. Control

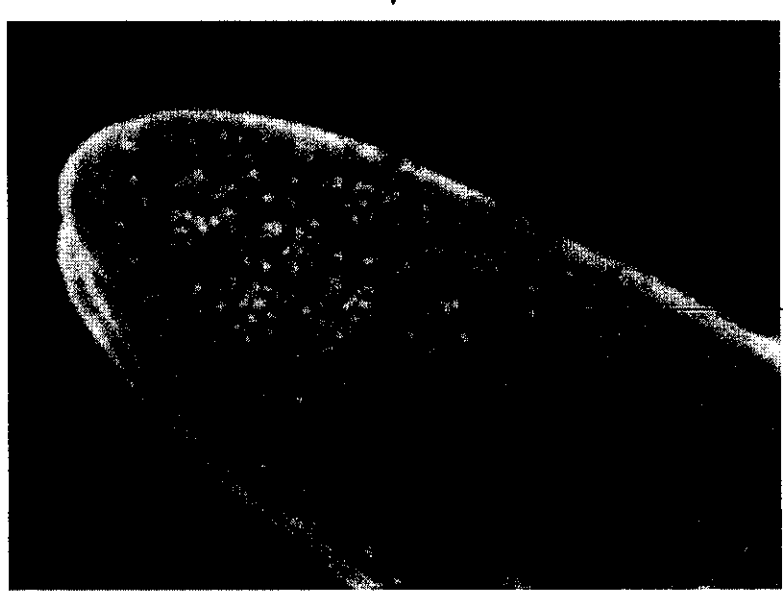


Fig no. 5. 8: *Pleurosigma* sp - Au Treated

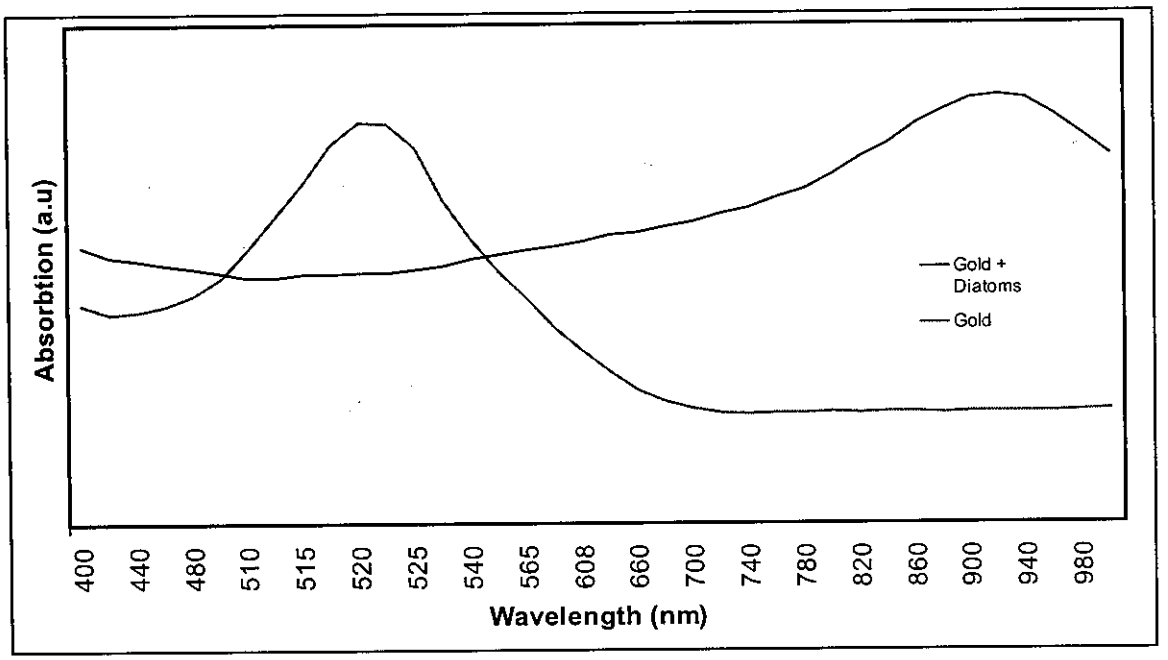


Fig no. 5. 9: UV- VIS Spectroscopy of Gold- Silica Nanocomposites

In the UV – Vis studies the chemically synthesized gold nanoparticles shows the peak at 520 nm, which is the characteristic feature for the gold nanoparticles. Where as in the case of gold nanoparticles synthesized along with diatoms shows the peak at 920 nm, which is near infrared region, which is shown in (Fig no. 5.9). Thus, the peak has been shifted from visible range to near infrared region and this shifting confirms the gold nanoparticles were attached to the silica shell of the diatom.

In the scanning electron microscope (Fig no. 5.5) and optical microscope (Fig no. 5.3), the surface of the diatoms is analyzed and a detailed study is done. The control and gold nanoparticles bind diatoms were observed and studied carefully and notable difference was found out. In the case of *Navicula* sp.

(Fig no. 5.5) the control organism was observed with the clean frustules structure. In the case of the gold nanoparticles bind diatoms the bright coloured nanoparticles were found to be attached throughout the surface of the silica shell of diatom and frustules uniformly which is shown in (Fig no. 5.6) In case of the *Pleurosigma* sp. also the gold nanoparticles bind diatoms, the surface was characterized with uniformly bind gold nanoparticles as shown in (Fig no. 5.8). In the optical microscopic studies on the surface of *Amphora* sp it is more interesting observation. The grooves and frustules of the organisms were stuffed with the gold nanoparticles and this give a pinkish colour to the diatom when compared to the colourless control *Amphora sp* as shown in (Fig no. 5.3). After many washing also it retained pinkish colour which confirms the gold nanoparticles bind strongly to the silica shell of the diatoms as in the (Fig no. 5.4).

The photoluminescence (PL) of Gold is generally attributed to electronic transition between the upper d band and conduction sp band . Au surface are known to exhibit poor luminescence efficiency and it is scarcely used for investigate surface properties (Rechberger *et al.*, 2003). As size decreases the spacing between discrete states in each band increases leading to blue shift in fluorescence (Mona *et al.*, 2000). A remarkable effect has been made to improve properties of the gold nanoparticles for the optoelectronics applications and is well established that the PL of the noble metals nanoparticles originates from surface

plasmon resonance and thus have much promises in many of the biological applications.

Due to the appearance of well stabilized and bind gold nanoparticles on the surface of the diatoms and the shift of the near infrared region it have great opportunities to use in the diagnosis purposes which excites and releases in the near infrared region. This work put lights to extra detailed study in the further optical studies like flourescent studies can be done in detail which promises a novel and toxic free method for the bioimaging purposes and diagnosis for many dreadful diseases like cancer.

CONCLUSION

5. CONCLUSION

Gold nanoparticles doped biocompatible silica shell having enhanced optical properties and using its fluorescent properties can be used for diagnosis purpose. It is a promising prospective candidate for bioimaging in future technology. The intricate structure of diatoms frustules offers a lager surface area respect to a planar glass slide and to silica microbead so that, beside the low cost material, better performances in biotechnological applications could be foreseen. These results could also push on the adoption of diatoms as natural devices in the micro and nano technological world.

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