CHAPTER 1
GENERAL INTRODUCTION
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Over the past few years a little word ‘Nano’ has conjured up the speculation about a seismic shift in almost every aspects of science and engineering with implications for ethics, economics, international relations, day-to-day life and even Humanity’s conception of its place in the universe. ‘Nano’ seems to stir up popular, political and media debate in the same way that the space travel and internet did in their respective heydays. Despite of the buzz and activity ‘Nano’ has moved from the world of future to the world of present. The Innovations of nano related fields have already sparked a flurry of commercial inventions. [1] But technically what is ‘Nano’? The word ‘Nano’ originates from the Greek word meaning ‘dwarf’.

1.1. NANOTECHNOLOGY

On a meter scale ‘a nanometer’ is only one billionth \((10^{-9})\) of a metre which is only a few lengths of hydrogen atoms or about a hundredth of the human hair. ‘Nanotechnology’ involves all the research and technology development at the atomic, molecular or macromolecular levels in the lengths of \(1–100\) nm range, to provide a fundamental understanding of the phenomena and materials at the nanoscale and to create and use structures, devices and systems that have novel properties and functions because of their small or intermediate size. The U.S. National ‘Nanotechnology’ initiatives definition includes anything smaller than 100 nanometer sizes with Novel properties is termed as ‘Nanotechnology’. [2]

1.1.1. BRIEF HISTORY

Nanotechnology dates back to medieval period when the first nanotechnologist was the glass worker. The color of the stained glass windows of the medieval and Victorian churches and the glazes found on the ancient pottery were due to the nanoscale properties of some of the materials used. But the concept of Nanotechnology in the present era is attributed to Nobel Laureate Richard
Feynman who introduced it in his famous lecture delivered in 1959 “There’s plenty of room at the bottom” which was later published in 1960: “The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom.” But the term ‘Nanotechnology’ was first coined by Tokyo Science University Professor Norio Taniguchi in 1974. Very little information is available other than his cited references of two of his books namely: “Nanotechnology: integrated processing systems for ultra-precision and ultra-fine products” and “On the basic concept of Nanotechnology”. [3] The term ‘Nanotechnology’ was not used again until 1981 when Eric Drexler, who was unaware of Taniguchi’s prior use of the term, published his first paper on nanotechnology in 1981. If Feynman was the philosopher then Dexler was the prophet. Feynman’s definition was expanded by Dexler in his book “Engines of Creation, the Coming Era of Nanotechnology” in 1986. To this in 1981 Binnig and Rohrer’s were the first to see atom by atom using their scanning tunneling microscope making ‘Nanotechnology’ possible. [4]

Thus nanotechnology is reported as a technology of building materials at the $10^{-9}$ level. So is it same as chemistry? The answer is a big ‘NO’. Then how is it different? Nanotechnology is not synonymous with chemistry, since it is more specific and concerned with observing atoms and molecules and manipulating them through visual observation at the nanoscale level. However, it may eventually encompass all of chemistry and a large part of physics and molecular biology. [4] When it comes to nanoscale all things do not simply scale down in proportion and there are problems that the materials stick together by molecular attractions (Van der Waals). Here the laws of Quantum mechanics, chemistry and physics would come into question. [3]

1.1.2. NANOFABRICATION TECHNIQUES

Two main techniques are used in Nanotechnology: One is a “Bottom up technique” where materials and devices are built up atom by atom, the other a “top-down technique” where they are synthesized or constructed by removing the existing material from larger entities.
1.1.2.a. **TOP DOWN APPROACHES**

In this technique a bulk material is reduced in size to nanoscale pattern using lithography, mechanical and chemical manipulation tools. Some of the lithographic techniques used are Atomic layer deposition (ALD), Electron beam lithography (EBL), Dip pen nanolithography (DPN), Nanoelectromechanical systems (NEMS).

Atomic layer deposition (ALD) is an advanced thin film growth technique in which the method relies on its unique growth process where alternate, self limiting surface reactions of the precursors, separated by inert gas purging, form a growth cycle where upon thin, up to one atomic layer of the material is grown. Precise thickness control can be achieved by repeating the growth cycle as desired number of times. [6]

Electron beam lithography (EBL) is a specialized technique used for creating integrated circuits at the nanoscale using a beam of electrons to generate patterns on the surface. This technique is use widely due to the speed of processing.

Dip pen nanolithography (DPN) is direct-write soft lithographic technique that is used to create nanostructures on a substrate of interest by delivering collections of molecules via capillary transport from an Atomic force Microscope (AFM) tip to a surface. This technique is a single step process of creating nanostructures on a surface.

NEMS are made of electromechanical devices that have critical dimensions from hundreds to a few nanometers. The interesting properties of the NEMS devices typically arise from the behaviour of the active parts, which in most cases are in the forms of cantilever or doubly clamped beams with dimensions at nanometer scale. In the fabrication process via top-down approach manufacturing of NEMS use submicron lithographic techniques, such as electron-beam lithography to fabricate the nanoscale materials, either thin films or bulk
substrates. But this technique is size limited by facts such as the resolution of electron beam lithography, etching-induced roughness and synthesis constrains in epitaxially grown substrates.

[7]

1.1.2.b. BOTTOM UP APPROACHES

The bottom up technique builds or grows larger structures atom by atom or molecule by molecule. [3] These techniques include Self assembly, Positional assembly, and Chemical synthesis.

Self assembly is a reversible process in which pre-existing parts or disordered components of a pre-existing system form structures of patterns. For example the most well studied subfield of self assembly is molecular self assembly. Molecular self assembly is the assembly of molecules without guidance or management from an outside source.

Positional assembly is highly precise forms of self assembly in which objects, device and systems are build on a molecular scale using automated processes in which the components that carry out the construction process would follow programmed paths.

In chemical synthesis the synthesis is carried out either under kinetic control condition or thermodynamic control condition. Though these factors are present in any synthesis process we are able to manipulate the experimental conditions to favour a preferred product whether it is the kinetic product or whether it is the thermodynamic product. Although the chemical synthesis have been studied and used in industrial processes for centuries, the conscious and deliberate use of nanomaterials as precursor materials, substrates, or moderators in reactions to synthesize new materials is a relatively a new approach. [5]

1.1.3. PRESENT TRENDS

The recent trends in the development and characterisation of nanomaterials include the integration of various disciplines of physics, chemistry, material science and engineering and biology
as well as toward the development of multifunctional materials compatible with increasingly complex environments. These trends fall into

(a) Integrations of organic and biological materials with nanoscale inorganic and polymeric materials (biomaterials).

(b) Incorporation of nanoscale components into materials to be improved functionally (nanocomposites).

(c) Use of “top-down” (e.g. lithography) and “bottom-up” (e.g. self-assembly) processing approaches sometimes in combination, to produce novel nanoscale structures (functional nanostructures). [8]

1.2. NANOPARTICLES

![Fig 1-1. Comparison of Nanometer sizes](image)

The definition of nanoparticles differs depending upon the materials, fields and applications concerned. In the narrower sense, they are regarded as the particles smaller than 10 - 20 nm, where
the physical properties of solid materials themselves would drastically change. On the other hand, the particles in the three digit range of nanometer from 1nm to 1 micrometer could also be called as nanoparticles. In many cases, the particles from 1 to 100 nm are generally called as nanoparticles, but here they will be regarded as the particles smaller than those called conventionally submicron particles and concretely less than the wavelength of visible light (its lower limit is about 400 nm) as a measure, which need to be treated differently from the submicron particles. [9]

There are two features of nanoparticles which make them so unique. Firstly the bonding state of the atoms or molecules constructing the particles where as secondly is the specific surface area making its properties change in solution state increasing the reaction rates.[9]

1.2.1. CLASSIFICATION OF NANOPARTICLES

Nanoparticles are generally classified based on their dimensionality, morphology, composition, uniformity and agglomeration. Controlling the morphologies of nanoparticles is of key importance for exploiting their properties for their use in several emerging technologies. Flatness, sphericity and aspect ratio are the characteristics which are taken into account.

Free particles can be composed of single material or be a composite of several materials. Nanoparticles found in nature are often agglomeration of materials with various compositions where as pure single composition materials can be easily be synthesized. Nanomaterials are also classified in terms of their chemical composition, such as nanometal, nanometal oxides, nanocarbon (eg. Carbon nanofiber, carbon nanotubes, graphite nanoplatelets) and nanoquantum dots. [11]
Fig 1-2(a): Classification of nanomaterials dimensions, morphology, composition, uniformity and agglomeration state and (b) classification based on the nanomaterial (discrete, surface and bulk structures).
1.2.1.a. METAL NANOPARTICLES

Nobel metal nanoparticles are attracting a huge interest due to their fabulous properties and diversity of applications. Although it has been known since prehistoric times and their applications were widely made then, the understanding of the nanoscale phenomena is a recent exploration. Tunability of properties by varying sizes, shapes and composition or local environment presents them with unusual capabilities. By manipulating the chemical composition of the materials at the nanoscale their electrical, chemical, optical and other properties can be manipulated precisely. [12]

The most important and interesting property of gold nanoparticles is their optical properties due to their interaction with light. In the presence of the oscillating electromagnetic field of the light, the free electrons of the metal nanoparticles undergo an oscillation with respect to the metal lattice. This process is resonant at a particular frequency of the light and is termed as the localized surface plasmon resonance (LSPR). After absorption, the surface plasmon decays radiatively resulting in light scattering or non-radiatively by converting the absorbed light into heat. Thus for gold nano spheres with particle size around 10 nm in diameter have a strong absorption maximum around 520 nm in aqueous solution due to their LSPR. These nano spheres show a stokes shift with an increase in the nano sphere size due to the electromagnetic retardation in larger particles. The surface plasmon absorption changes with the shape of the nanomaterials. The rod shaped nanoparticles have two resonances: one due to plasmon oscillation along the nano rod short axis and another due to plasmon oscillation along the long axis, which depends strongly on the nano rod aspect ratio, that is, length to width ratio [13].

Gold nanoparticles have unique properties, providing a high surface area to volume ratio. Moreover, the gold surface offers a unique opportunity to conjugate ligands such as
oligonucleotides, proteins, and antibodies containing functional groups such as thiols, mercaptans, phosphines, and amines, which demonstrates a strong affinity for gold surface.

Similar to gold nanoshells, gold nanocages represent a novel class of nanostructures that are hollow porous gold nanoparticles that absorb light in the near infrared range. They have applications in drug delivery and/or controlled drug release. Furthermore, the hollow interiors can host small objects such as magnetic nanoparticles to construct multifunctional hybrid nanostructures diagnostic imaging and therapy.

Ionic silver has a long history and was initially used to stain the glass for yellow. Currently, there is also an effort to incorporate silver nanoparticles into a wide range of medical devices, including bone cement, surgical instruments, surgical masks, etc. Moreover, it has also been shown that ionic silver, in the right quantities, is suitable in treating wounds. In fact, silver nanoparticles are now replacing silver sulfadiazine as an effective agent in the treatment of wounds [14].

Copper is a highly conductive, much cheaper, and industrially widely used material, possessing a valence shell electron structure similar to the other two coinage metals. Furthermore, it is unique with the chemical reactivity capable of serving as precursors for the fabrication of conductive structures by ink-jet printing or forming CuInSe$_2$ or CuIn$_x$Ga$_{1-x}$Se$_2$ semiconducting nanomaterials for photo detectors and photo voltaics. Nevertheless, over the years, fabrication of Cu nanoparticles has received less attention as compared to that of Au and Ag ones and is still open for more intensive investigations [15].

Magnetic nanoparticles have attracted increasing interest among researchers of various fields due to their promising applications in high-density magnetic recording,
medical and biomedical, such as magnetic resonance imaging (MRI), cell and DNA separation, drug delivery, gene cloning, and hyperthermia for cancer therapy. Co-nanocrystals display a wealth of size-dependent structural, magnetic, electronic, and catalytic properties. [15]

1.2.1.b. METAL OXIDE NANOPARTICLES

Metal elements are able to form large diversity of oxide compounds [16]. These oxides exhibit a number of structural geometries as well as behave as metallic, semiconductor or insulators thereby making it useful in the fabrications of microelectronic circuits, sensors, piezoelectric devices, fuel cells, coatings for the passivation of surfaces against corrosion, and as catalysts. However to obtain metal oxides as nanoscale materials with well defined shape, size and composition, traditional solid state synthesis based methods are not suitable. High temperature processes, soft chemistry routes, and in particular sol-gel procedures, feature advantages such as the possibility of obtaining meta stable materials, achieving superior purity and compositional homogeneity of the products at moderate temperatures with simple laboratory equipment and influencing the particle morphology during the chemical transformation of the molecular precursor to final oxidic network. [17]

Oxide nanoparticles can exhibit unique physical and chemical properties due to their limited size and a high density of corner or edge surface sites. Particle size is expected to influence three important groups of basic properties. The first one comprises the structural characteristics namely lattice symmetry and cell parameters. [18] Changes in thermodynamic stability associate with size can induce modification of cell parameters and cause structural transformations. Size induced structural distortions associated with changes in cell parameters have been observed in nanoparticles such as ZrO$_2$ [19] and
also the phases with low stability in bulk materials is seen to become very stable in nanostructures in cases of TiO$_2$. [20]

Second important size related effect is the electronic properties of the metal oxide. Theoretical studies for oxides showed a redistribution of charge when going from large periodic structures to small clusters or aggregates which could have been small for ionic solids while significantly large for covalent ones. The degree of ionicity or covalency in a metal oxygen bond can however strongly depend on size in systems with partial ionic or covalent character, an increase in the ionic component to the metal oxygen bond in parallel to the size decreasing has been proposed. [21] A decrease in the average size of an oxide particle in fact changes the magnitude of the band gap [22, 23] with strong influence in the conductivity and chemical reactivity [24, 25].

1.2.1.c. CARBON NANOMATERIALS – GRAPHENE, GRAPHENE OXIDE AND REDUCED GRAPHENEOXIDE

Carbon is a unique element due to its ability to form a variety of nanomaterials ranging from Zero dimensional fullerenes to one dimensional conducting and semiconducting carbon nanotubes and two dimensional semi metallic graphene. Carbon is a tetravalent element (atomic configuration: 1s$^2$, 2s$^2$, 2p$_x^1$ and 2p$_y^1$). When forming bonds with other atoms carbon promotes one of the 2s electron into the empty 2p$_z$ orbital, resulting in the formation of hybrid orbital’s. In graphite only two of the three 2p orbital partake in the hybridization forming three sp$^2$ orbitals. The sp$^2$ orbitals are oriented perpendicular to the remaining 2p-orbital, therefore lying symmetrically in the X-Y plane at 120° angles. The sp$^2$ carbon atoms form covalent in-plane bonds affecting the planar hexagonal honeycomb structure of the graphite. In graphene the in-plane σ-bonds (615 kJ/mol) are even stronger than the C-C bonds in sp$^3$- hybridized diamond (345 kJ/mol)
but the interplanar π-bonds formed by the remaining 2p-orbitals, have a significantly lower binding energy, leading to an easy shearing of graphite along the layer plane. [26]

![2D hexagonal lattice of graphene in real space with basis a₁ and a₂.](image)

Fig 1-3: 2D hexagonal lattice of graphene in real space with basis $a_1$ and $a_2$.

In fig 1-3, the unit cell is highlighted in grey. It contains two nonequivalent carbon atoms A and B each of which span a triangular sublattice as indicated with black and white atoms, respectively. An arm chair and a zig-zag edge are highlighted in grey.

A single layer of graphite or graphene layer has a lattice constant $a = \sqrt{3} a_0$ where $a_0 = 1.42 \text{ Å}$ is the nearest neighbour interatomic distance. The interplanar distance between two adjacent graphene layers in stacked graphite is $3.35 \text{ Å}$. Graphene is incorrectly used for ultrathin graphite layers. It strictly refers to a quasi-two dimensional isolated monolayer of carbon atoms that are arranged in a hexagonal lattice. Only single layer graphene (SLG) and Bilayer graphene (BLG) are zero –gap semiconductors with only a single type of electrons and holes respectively. In the case of few layer graphene (FLG, 3 to < 10 layers) the conduction and valence bands start to develop and several charge carriers appear. The thicker graphene structures are considered as thin films of graphite. Graphene is a zero-gap semiconductor since the conduction and valence bands touch at the Dirac points and exhibit a linear dispersion.
The charge carriers are massless, which affects extreme intrinsic carrier mobility. This makes graphene a promising candidate for the applications in new generation molecular electronics such as graphene based interconnects and field effect transistors.

Graphene was first isolated from graphite via mechanical exfoliation and was found to be stable on a supporting substrate under ambient conditions. Later studies showed that in-plane and out of plane distortions of graphene lattice were essential for the structural stability of graphene. These corrugations can effectively limit the electronic properties of graphene.

![Proposed atomic model of graphite oxide.](image)

Graphitic regions are interrupted by strongly oxidized regions where functional hydroxyl and epoxide groups are located.

Graphene oxide (GO) and reduced graphene oxide (rGO) have been studied intensively as alternative materials to graphene. Large strains are induced by the functional hydroxyl and epoxide groups present on both sides of the GO layers thereby creating fundamental structural and topographical differences between chemically modified and mechanically cleaved graphene. [27]
1.2.2. METHOD OF SYNTHESIS OF NANOPARTICLES – BOTTOM-UP APPROACH - CHEMICAL SYNTHESIS

Among the two major approaches of bottom-up and top-down techniques used, the chemical synthesis of nanoparticles fall under the bottom up category. The fig 1-5 given indicates the different Bottom-up techniques used for nanoparticle synthesis.

Chemical methods tend to control the clustering of atoms/molecules at nanoscale range. The wet chemical methods are most popular over the conventional methods as they are highly reliable and cost effective, and they allow a much more rigorous control of the shape and size of the nanoparticles and agglomeration of the resulting particles can be alleviated by functionalization with different capping ligands. The wet chemical methods can yield colloidal solutions with a wide range of particle sizes on the gram scale in combination with high reproducibility.
Some of the wet chemical methods are

1.2.2.a. SOL-GEL METHOD

The sol-gel process is defined as the method used for the production of inorganic oxides such as ceramics with an intermediate stage including a sol and/or a gel state, either from colloidal dispersion or from metal alkoxides. [28] However this process no longer stays solely for oxides but some components such as nitrides and sulfides as well as the recent synthesis of hybrid organic-inorganic materials also fall in to this category. The fig 1-7 below shows the general outline of the sol-gel process.

There are size important steps in sol-gel synthesis

i. **Hydrolysis:** The process may start with a mixture of metal alkoxide and water in a solvent (usually alcohol) at the ambient or a slightly elevated temperature. Acid or base catalysts are added to speed up the reaction

ii. **Polymerization:** This step involves condensation of adjacent molecules where in H₂O and alcohol is eliminated and metal oxide linkages are formed. Polymeric networks grow to colloidal dimensions in the liquid (sol state).

iii. **Gelation:** In this step the polymeric networks links up to form a three-dimensional network throughout the liquid. The system becomes somewhat rigid, characteristic of a
gel, on removing the solvent from the sol. Solvent as well as water and alcohol molecules, however, remain inside the pores of the gel. Aggregation of smaller polymeric units to the main network progressively continues on aging the gel.

iv. **Drying:** Water and alcohol are removed at moderate temperature (< 470K) leaving a hydroxylated metal oxide with residual organic content. In case of a high surface area aero gel powder of low bulk density the solvent is removed super critically.

v. **Dehydration:** This step is carried out between 670 and 1070 K to drive off the organic residues and chemically bound water, yielding a glassy metal oxide with up to 20-30 % micro porosity.

vi. **Densification:** Temperatures in excess of 1270K are used to form the dense oxide product.[29]
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Sol-gel method permits the production of new hybrid organic-inorganic materials which do not exist naturally. It avoids any pollution by the eventual dispersion of dust. The most outstanding advantage is the production of mixed oxide systems in which chemical homogeneity of the various elements can be controlled down to the atomic level. But the greatest limitation of this method is that of the cost of the precursors to be used. [28]

1.2.2.b. PRECIPITATION SYNTHESIS

Precipitation synthesis and co-precipitation synthesis techniques are sometimes considered as a side branch of sol-gel processing. The chemical reactions concerned are as the same as occurring in sol-gel synthesis which leads to the production of the colloidal particles, but can also be dispersed into a stable sol. [28]

In a solution when reactive species is supersaturated, a new phase nucleates homogenously out of this solution. Then particles grow via molecular addition, which acts to decrease the super-saturation. When the concentration of soluble material drops below the critical level, nucleation stops and particles continue to grow by molecular addition until the equilibrium concentration of the precipitating species is reached. Uniformity of size distribution is achieved through a short nucleation period that generates all of the particles seen at the end of the reaction followed by a self-sharpening growth process where smaller particles grow more rapidly than larger ones, thereby narrow particle size distribution is maintained. [31]

1.2.2.c. SOLVOTHERMAL / HYDROTHERMAL REACTION

Solvothermal reactions are used for preparing micro or nanoparticles with different morphologies. This technique finds an increasing number of applications in nanotechnology and offers a unique means of making highly functional materials having potential applications in sensors, separation and catalysis, molecular devices and spintronics. The process is defined as a chemical reaction in a closed system in the presence of a solvent (aqueous and non-aqueous
solvent) at a temperature higher than that of the boiling point of such a solvent. As a result high pressure (< 1bar) is involved depending on the selected temperatures which can be in sub or supercritical domains. When the solvent is water the technology is termed as hydrothermal. Besides water when solvents such as ammonia (ammonothermal synthesis), glycols (glycothermal), alcohols (alcothermal) are the most important solvothermal reaction media. [30]

Hydrothermal process is used for the preparation of hydroxides, oxy-hydroxide or oxides and in case of non oxide metals to be developed the non-aqueous solvents are used.

**Step 1, Hydrolysis:** $ML_x + xOH^- \rightarrow M(OH)_x + xL^- \quad Eq(1-1)$

**Step 2, Dehydration:** $M(OH)_x \rightarrow MO_{x/2} + x/2 H_2O \quad Eq(1-2)$

There are two parameters involved in the solvothermal reactions – chemical and thermodynamical. Based on the analysis of the chemical factors involved in the solvothermal process the reactions can be classified into five types

(i) Oxidation-reduction

(ii) Hydrolysis

(iii) Thermolysis

(iv) Complex formation

(v) Metathesis reactions

Solvothermal reactions are characterized based on the nature of the reagents and solvents, temperature and pressure. These reactions are used to prepare specific materials such as inorganic (oxides-nitrides, nitrides-halides etc), inorganic/organic, inorganic/biological frameworks thus leading to the production and investigation of newer nanomaterials. The solvothermal synthesis is used to control grain size, particle morphology, crystalline phase and surface chemistry by regulating sol composition, reaction temperature and pressure, nature of solvent, additives and aging time. The particles prepared are reported to have large surface
area, smaller particle size and were more stable than those obtained by other methods such as sol-gel. [32]

1.2.3. SYNTHESIS OF GRAPHENE/GRAPHENE OXIDE

SLG and FG are synthesized by several methods. The synthesis procedure can be broadly classified into exfoliation, chemical vapour deposition (CVD), arc discharge and reduction of graphene oxide. [26]

1.2.3.a. EXFOLIATION

Stacking of sheets in graphite is the result of overlap of partially filled $p_z$ or $\pi$ orbital perpendicular to the plane of the sheet (involving Vander Waals forces). Exfoliation is the reverse of stacking.

1.2.3.a.1 MECHANICAL EXFOLIATION

Graphene sheets of different thickness can indeed be obtained through mechanical exfoliation or by peeling off layers from graphitic materials such as highly ordered pyrolytic graphite (HOPG), single crystal graphite, or natural graphite. Peeling and manipulation of graphene sheets have been achieved through AFM and STM tips.[33-38] The mechanical exfoliation produces graphene of the highest quality (with least defects) but is limited due to the low productivity. Hence chemical exfoliation is preferred for bulk scale production

1.2.3.a.2 CHEMICAL EXFOLIATION

The process is two step procedure in which the first step is to increase the interlayer spacing, thereby reducing the interlayer van der Waal’s forces. This
is achieved by intercalating graphene to prepare graphene-intercalated compounds (GICs) [39]. These GICs are therefore exfoliated into graphene with single to few layers by rapid heating or sonication.

Hummers Method for Graphite Oxide: [40]

50 g of Sodium nitrate and 100 g of graphite are added to 2.3 L of concentrated sulphuric acid and cooled to 0°C using an ice-bath. Vigorous agitation is applied as 300g to potassium permanganate is added to the mixture at a rate such that the temperature does not reach above 20°C. The ice-bath is removed and the temperature rose to 35°C for 30 min at which point the mixture becomes pasty with a brown-grey colour. Next, 4.6 L of water is added, resulting in effervescence and a temperature rise to 98°C. After 15 min at 98°C, the solution is diluted with 14L of warm water and 3 % hydrogen peroxide to reduce permanganate and manganese dioxide to manganese sulfate. The solution is filtered whilst still warm to form a solid cake and then washed three with a total of 14 L of water. Remaining salt impurities were removed by treating with resinous anion and cation exchanges. A dry graphite oxide powder was finally obtained by centrifugation, followed by drying at 40°C over phosphorous pentoxide.

The oxidation of graphite increases the interlayer spacing from 3.7 to 9.5 Å and exfoliation resulting in SLG is achieved by simple ultrasonication in a DMF (dimethyl formamide) /water (9:1) mixture. The GO has a high density of functional groups and reduction needs to be carried out to obtain graphene like properties.
Exfoliation is also carried out by giving thermal shock to the acid-intercalated graphite oxide using microwaves. [41] When irradiated in microwave oven, eddy currents are generated because of the stratified structure of GO yielding high temperatures by Joules heating. Decomposition and gasification of the intercalated acids in graphite leads to a sudden increase in interlayer spacing and thereby reduces van der Waals interaction. Further sonication yields and SLG and FG sheets.

1.2.3.b. CHEMICAL REDUCTION OF GRAPHENE OXIDE

As graphene is insoluble in water and will aggregate, the reduction of graphene oxide to graphene in aqueous solutions was one of the initial challenges. But later graphene oxide was converted to graphene by using hydrazine [42] by the so called modified Hummers method as given below.

Graphite oxide was dispersed in water at 0.05 % wt by 30 min of sonication. Thirty minutes of 3000 rpm centrifugation was then applied to remove aggregates, leaving a homogeneous graphene oxide solution, 5 ml of the graphene oxide solution was mixed with 5 ml of water, 5 μl of hydrazine (35%wt in water) and 35 μl of ammonia solution (28%wt in water), then shaken for a few minutes and placed in a water bath at 95°C for 1 h.

The reduction is achieved with hydrazine monohydrate to give well-dispersed SLG sheets [43-44]. Some other reducing agents used include NaBH₄ [45], Phenyl hydrazine [46], hydroxylamine [47], glucose [48], ascorbic acid [49], hydroquinone [50], alkaline solutions [51] and pyrrole [52]. The exfoliation via chemical method results in graphene sheets with high wrinkles and defects as a result these sheets do not collapse back to graphite but remain as highly agglomerated graphene sheets.
Thermal expansion of graphite oxide can be used to both exfoliate and produce functionalized graphene sheets. At Critical temperature of 550°C the decomposition rate of epoxy and hydroxyl sites of graphene oxide becomes larger than the rate of diffusion of the evolved gases and this causes a buildup of pressure. [53] The pressure becomes so great that it overcomes the van der Waals forces that bind the graphene sheets together and exfoliation occurs. Thermal exfoliation and reduction of graphite oxide also produce good-quality graphene generally referred to as reduced graphene oxide (RGO). The resulting functionalized graphene sheets with thickness between 1 and 3 nm and diameters between 0.3 and 2 microns. The functionalized graphene sheets were shown to be electrically conducting, indicating they were not graphene oxide which was insulating. [54]

Electrochemical method is another way of reducing graphene in a large scale. [55-57] The reduction initiates at -0.8 V and is completed by -1.5 V, with the formation of black precipitate onto the bare graphite electrode. Other reduction methods include photo thermal and photochemical reduction of GO. SLG to FG were obtained by reducing GO using UV radiation[58] and RGO were prepared via photo thermal reduction by using xenon flash at ambient conditions.[59] Nanosecond laser pulsed of KrF eximer laser or 335 and 532 nm were shown to effectively reduce GO [60]. Electron-beam-induced reduction of GO has also been reported. [61]

1.2.4. CHARACTERISATION TECHNIQUES OF NANOPARTICLES

A better understanding and various potential applications increasingly demand the ability and instrumentation to observe measure and manipulate the individual nanomaterials and nanostructures. Characterization and manipulation of individual nanostructures requires not only the extreme sensitivity and accuracy, but also the atomic level resolution. Therefore, various microscopies play a central role in the characterization
and measurements of various nanostructured materials. Not only is the miniaturization of the instrument is the challenge, the new physical properties and short range forces not noticeable in macroscopic level may have significant impacts in the nanometer scale. Hence the novel tools and instruments is also one of the greatest challenges in nanotechnology.

1.2.4.a. STRUCTURAL CHARACTERISATION

Structural Characterization of nanomaterials and nanostructures has been largely based on the surface analysis techniques and conventional characterization methods developed for bulk materials.

1.2.4.a.1 X-RAY DIFFRACTION (XRD)

XRD has been used for the determination of crystallinity, crystal structures and lattice constants of nanoparticles, nanowires and thin films. In XRD, a collimated beam of X-rays, with a wavelength typically ranging from 0.7 to 2 Å, is incident on the specimen and is diffracted by the crystalline phases in the specimen according to Bragg’s law

\[ \lambda = 2d \sin \theta \]

Where \( d \) is the spacing between atomic planes

\( \lambda \) is the X-ray wavelength

\( \theta \) is the half of the diffraction angle

The intensity of the diffracted X-rays is a function of the diffraction angle 2\( \theta \) and the specimen’s orientation. XRD is a non-destructive technique and does not require elaborated sample preparation. Using the Scherrer’s formula [62] the crystallite size \( D \) can be estimated using the peak width as
\[ D = \frac{k \lambda}{B \cos \theta_B} \quad \text{Eq (1-4)} \]

Where B is the full width of half maximum (FWHM)

\[ \theta_B \] is the diffraction angle

k is the Scherrer’s constant of the order of the unity for usual crystals.

Due to Twinning the information obtained from XRD for nanomaterials are often different from the true particle sizes. But the technique gives collective information of the particle sizes and usually requires a sizable amount of powder since the diffraction intensity is dependent on the amount of sample analyzed. Hence for high –Z materials XRD is preferred.

**1.2.4.a.2 ELECTRON MICROSCOPY**

Louis De Broglie first asserted that matter like light has both particle and wave characteristics. By comparison, subatomic particles have wavelengths comparable to the size of the atom. In particular the electrons when accelerated at 100kV have a wavelength of 0.037Å. This is shorter than the wavelengths of high energy radiation sources such as X-rays (1.54 Å for Cu Kα X-rays) or neutrons (ca. 1nm). The ability to focus the extremely small incident wavelengths of energetic electrons results in an unprecedented spatial resolution compared to optical based microscopy techniques. In order for us to observe the interesting features appropriate magnification must be used.

There are two types of scattering interactions that are possible when an electron beam impinges on atoms of the sample. Elastic scattering which results in a large angle deflection of electron beam with little or no energy loss and small angle deflections with significant energy loss – inelastic scattering.

Two types of electron microscopes include – scanning electron microscope (SEM) and transmission electron microscope (TEM) having magnifications of 100-300 K and 1.5-
2 M respectively. TEM works in transmission mode, the intensity from an electron source after it passed through a transparent sample and in contrast SEM features the scanning of an electron beam over selected regions of an opaque sample.

In a typical SEM, source of electrons is focused into a beam, with a very fine spot size of ~ 5nm and having energy ranging from a few hundred eV to 50 KeV that is rastered over the surface of the specimen by deflection coils. As the electrons strike and penetrate the surface, a number of interactions occur that result in the emission of electrons and photons from the sample and SEM images are produced by collecting the emitted electrons on the cathode ray tube (CRT). [62] SEM not only provides the image of the morphology and microstructures of the bulk and nanostructured materials and device, but also provides detailed information of chemical composition and distribution.

Fig 1-8: Schematic of the major components of (a) Transmission electron microscope (TEM) and (b) scanning electron microscope (SEM).
A TEM is analogous to a slide projector with illumination from an electron beam rather than light. When an electron beam is impinged upon a sample, a black and white TEM image is formed from the passage of some electrons through the sample untouched, alongside the combination of interactions between other electrons and sample atoms. If the undiffracted beam is selected to form the image, it is referred to as bright-field imaging and in contrast the selection of strongly diffracting regions of the sample, which would appear brighter than the transmitted beam, is known as dark-field imaging.

TEM is also considered as complementary to the conventional crystallographic methods such as X-ray diffraction. When electrons interact with a crystalline solid, comprising a regularly spaced three dimensional array of atoms, the incoming beam is diffracted at specific angles predicted by the Bragg equation (Eq 1-3). In order to understand the information contained within the diffraction pattern of a crystal lattice it is necessary to construct a secondary lattice known as reciprocal lattice. To determine the lattice planes which give rise to diffraction a geometrical construct known as Ewald Sphere is used. When reciprocal lattice points intersect the Ewald sphere it results in few sets of planes that give rise to diffracted beams. As a result, a single crystal gives a few diffraction spots which are sharp, a polycrystalline sample yield many closely spaced diffraction spots and an amorphous sample gives rise to diffused rings. Information about lattice parameters and atomic positions in crystal can be obtained by analysing the in situ electron diffraction pattern from a specimen size of > 400 nm ( > 100 nm for a field emission source) This technique is denoted as Selected area electron diffraction (SAED). Therefore structural information about individual nanocrystal is elucidated by TEM/SAED technique. [63]
1.2.4.a.3 ATOMIC FORCE MICROSCOPY

It is one of the primary forms of Scanning probe microscopy in which the tip may interact with the sample surface through either contact or noncontact modes. AFM is used to provide an image of the surface topography and it is carried out under ambient temperatures/pressures, or even in liquids [64] or may be performed in an UHV chamber (10^{-11} Torr) [65-67] in order to prevent sample contamination, oxidation of the surface, or monitor the deposition of sputtered thin films in real time.

AFM measures the forces between the tip and surface, which depends on the nature of the sample, the probe tip and the distance between them [68]. The most common operating modes of AFM are contact, noncontact and tapping. In contact-mode AFM, a repulsive force exists between the sample and tip (10^{-9} N) and the piezoelectric response of the cantilever either rises or lowers the tip to maintain a constant force. In noncontact AFM, the attractive Van der Waal forces between tip and surface are monitored. This mode overcomes the frictional and adhesive forces between the tip and sample by hovering the tip a few Angstroms above the surface.

In tapping mode AFM, the cantilever oscillation amplitude remains constant when not in contact with surface. The tip is then carefully moved downward until it gently taps the surface. As the tip passes over an elevated surface feature the cantilever has less room to oscillate and the amplitude of oscillation decreases. Tapping mode is most appropriate for soft samples such as organics, biomaterials etc.

1.2.4.a.4 DYNAMIC LIGHT SCATTERING (DLS)

A monochromatic light interacts with the particles undergoing Brownian motion in a fluid. The motion is size dependent (e.g., larger particles move more slowly
through the fluid) and this causes a shift in the frequency of the scattered light (Doppler shift). Larger the particles lower the velocities and hence lower the frequency shifts. Measuring the frequency shift provides the movement of the particles and their hydrodynamic size distribution. DLS measurements of nanoparticle hydrodynamic diameter can be used as a method for monitoring the stability of nanoparticle solution. When the stability of the nanoparticles is changing and aggregates/agglomerates form, the DLS spectrum of hydrodynamic diameter increases. DLS can also be used for determining the quality of nanoparticle dispersion by directly measuring the zeta potential, which is a measure of particle interaction. The particles will repel each other and resist the formation of clusters when their zeta potential is large above +30mV or below -30mV. [12]

1.2.4.b CHEMICAL CHARACTERISATION

Chemical characterization is used to determine the surface and interior atoms and compounds as well as their spatial distribution. These techniques generally group into various optical and electron spectroscopy.

1.2.4.b.1 OPTICAL SPECTROSCOPY- UV-VIS ABSORPTION

Band gaps of all semiconducting and insulating materials increases monotonically with decreasing size of the nanocrystallites. However, the band gap increases appreciably only in the domain of extremely small sizes when the size of the nanocrystals is reduced below the Bohr exciton radius, $a_B$ of the material. The band gap increase is most easily observed in the UV-Vis absorption spectra as the absorption edge shifts systematically to lower wavelengths. On the other hand UV absorption spectrum can also provide a qualitative indication of the size distribution. If the size distribution is
broad there would be a number of exciton peaks appearing at different energies corresponding to different sized nanocrystals as shown in fig 1-9. [69]

![UV-Vis absorption spectra of CdS nanocrystallites of various sizes](image)

**Fig 1-9:** UV-Vis absorption spectra of CdS nanocrystallites of various sizes

### 1.2.4.b.2 FOURIER TRANSFORM INFRA RED SPECTROSCOPY (FTIR)

FTIR stands for Fourier transform infrared which is the preferred method of infra red spectroscopy. Here IR radiation is passed through a sample. Some of the radiation is absorbed by the sample and some of it is passed through. The resulting spectra represent the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis. This method is widely used in organic synthesis, polymer science, food industry etc. The use of an interferometer in FTIR spectrometer improves the slow scanning process. [69-70]

### 1.2.4.b.3 RAMAN SPECTROSCOPY

Raman spectroscopy is based on vibrational technique and differs from infra red by an indirect coupling of high frequency radiation, such as visible light, with vibrations of chemical bonds. The spectrum is very sensitive to lengths,
strengths, and arrangement of chemical bonds in a material, but less sensitive to the chemical composition.

When an incident photon interacts with the chemical bond, the bond gets excited to a higher energy state. The reradiated frequency will be the same as that of the exciting light known as Rayleigh scattering. A small portion of the energy is transferred and results in exciting the vibration modes by Raman process, resulting in a reradiation with slightly lower frequency (smaller wave number) than that of the incident exciting light called stokes scattering. The vibrational energy is deducted by measuring the difference between the Raman line frequency and Rayleigh line frequency. There can also be Anti-stokes scattering resulting in Raman lines at higher frequencies which may be due to the thermal activation. Though Stokes and Anti –Stokes spectral lines appear as mirror images on opposite sides of the Rayleigh line the Stokes scattering spectra are mostly used since they are less temperature sensitive. Since Raman Effect is extremely weak highly intense monochromatic gas lasers are used as exciting light. The spectroscopy gives more structural characterization details than chemical analysis. [62]

1.2.4.b.4 X-RAY PHOTOELECTRON SPECTROSCOPY (XPS)

X-ray photoelectron spectroscopy (XPS) or electron spectroscopy for chemical analysis (ESCA) uses the phenomenon of photoelectric effect for surface analysis. This technique consists of the irradiation of sample with monochromatic X-rays (eg. Al Ka (1.487 keV), Mg Kα (1.254 keV), Ti Kα (2.040 keV)) which releases photoelectrons from the sample surface. Due to short free mean path (FMP) of the photoelectrons in the solid, the technique provides compositional information from only the top 1-5 nm of a sample.
Each atom has characteristic binding energies of their inner-shell electrons, referred to as absorption edges. Since the binding energy of an atom is altered by minute changes in its chemical environment (e.g., oxidation state, hybridization/geometry, etc), XPS provides both elemental quantification and details regarding the chemical environment of the surface atoms. [71] By detecting photoelectrons emitted from a surface at different emission angles, one obtains the energy of the electrons as a function of the momentum vector, \( k \). This is referred to as “band mapping” since this analysis probes the electronic structure of the crystalline materials. [72-73]

### 1.2.4.c ELECTRICAL CHARACTERISATION - CONDUCTIVITY – HALL PROBE

The conductivity \( \sigma \) (S/cm) is related to the specific resistance \( \rho \) (Ω cm)

\[
\sigma = \frac{1}{\rho} \quad \text{Eq (1-5)}
\]

The conductivity can be calculated from basic semiconductor properties in case of dominating electron conduction

\[
\sigma = q\mu n \quad \text{Eq (1-6)}
\]

With the elementary charge \( q \) (1.602 × 10\(^{-19}\) A s) the carrier mobility \( \mu \) (unit cm\(^2\)/V s) and the carrier density \( n \) (unit cm\(^{-3}\))

If both carrier types (electron and holes) are concerned, the conductivity is deducted from

\[
\sigma = q\mu_n n + q\mu_p p \quad \text{Eq (1-7)}
\]

The standard method for measuring the specific resistance of bulk materials and thin layers is the four point probe. By applying the method to bevelled surfaces, in-depth resistivity profiles can be deduced.
Fig 1-10: Four-point probe applied to a thin, infinitely extended layer of thickness $d$ and specific resistance $\rho$; the measure layer is isolated by a semi-insulating substrate.

A measurement head comprising four spring-loaded needles with equal distances $s$ ( $s = 1$ mm) is pressed onto the semiconductor sample with a pressure ensuring good near-ohmic contacts, but soft enough not to produce visible damage. The measurement current $I$ is fed via the outer needles through the sample. The voltage $V$ is measured between the inner needles. The specific resistance of the sample is

$$\rho = \frac{V}{I} c \text{------------------------Eq (1-8)}$$

With $c$, denoting a geometry dependent correction factor depending on the same dimensions and the needle.

The mobility $\mu$ of the majority carriers is extracted by a two-step procedure applying van der Pauw test structures. Firstly the specific resistance $\rho$ is measured; secondly the carrier concentration $n$ is determined. In a final step the mobility is deduced from the equation $\sigma = q\mu n$

$$\mu = \frac{\sigma}{qn} = \frac{1}{qn\rho} \text{------------------------Eq (1-9)}$$
The term $\left|\frac{1}{qn}\right|$ is the Hall constant, which is indicative of the carrier concentration $n$ within the test sample

$$\mu = \frac{R_H}{\rho} = R_H \sigma$$

Eq (1-10)

Due to the fact that the Hall Effect is polarity sensitive with respect to the conduction type, the sign of the Hall constant comprises the information of the dominating carrier type; negative sign – n type, positive sign – p type. [74]

1.2.4.d **OPTICAL CHARACTERISATION - ELLIPSMOMETRIC ANALYSIS**

It is an optical technique related to polarized light and commonly based on reflection. It finds application at surfaces where the optical properties of surfaces and surface films whether continuous or discontinuous can be determined.

An incident beam of linearly polarized light with electric filed $E$ can be resolved into two orthogonal components $E_{ix}$ and $E_{ip}$. The plane defined by the incident beam and the surface normal is called the plane of incidence (POI) and the incident beam after reflection from the surface has two components: $E_{rp}$ parallel to POI and $E_{rs}$ orthogonal to POI. [75]

![Image of imaging ellipsometry](image-url)

**Fig 1-11: Schematic of imaging ellipsometry**
Chapter 1 General Introduction

Imaging Ellipsometry also known as spectrometric Ellipsometry (SE) has become one of the most commonly used techniques for optical characterization of surfaces, interfaces and thin films particularly in the area of micro-and nano-electronics. SE has been and is used to measure film thickness and optical dielectric function of dielectrics, semiconductors and metallic thin films.

Ellipsometry is a non destructive, non invasive, contactless and fast technique that could be used even in-situ at the solid-liquid interface to monitor kinetics of biochemical reaction happening on the biosensor surface. [77] It measures the change of polarization state of linearly polarized light upon reflection from a sample, obtaining two independent parameters, ψ and Δ. Ellipsometry determines the complex amplitude ratio ρ defined as the ratio of the amplitude reflection coefficients r_p and r_s given as

\[ \rho = \frac{r_p}{r_s} = \tan(\psi).exp(i\Delta) \]  

Eq (1-11)

Where, p and s stands for p- and s- polarizations.

In SE the (ψ, Δ) is measured by changing the wavelength of light. In most cases the spectral range covers the ultraviolet/visible (UV/VIS) range but the infrared (IR) range has also been widely used.

Most advantage is precision in measurement. This technique is very sensitive to any changes of layer thicknesses even below 1 nm with a sensitivity of 0.1Å. It turns out that Ellipsometry is a promising technique for nanomaterial characterization. One of the major advantages of this technique is that the sample analysis can be performed in the ambient, liquid or vacuum environment. [75]
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The inherent disadvantage of Ellipsometry is that it requires an optical model that defines the number of constituents, the structure and morphology of each constituent and the overall structure and morphology of the sample. [74]

1.2.5. DEVICE CHARACTERISATION

1.2.5.a I-V CHARACTERISATION FOR SOLAR CELLS

Standard spectra is needed in solar cell research, development and marketing because the cell operations varies widely due to weather, season, time of the day and location. Solar simulators are used to duplicate the standard conditions.

The total power $P_{IN}$ per area impinging on a cell for a given photon spectrum $\Phi_0 (\lambda)$ is the integral of the incoming energy per time per area per bandwidth over the entire photon spectrum

i.e., $P_{IN} = \int_{\lambda}^{\lambda} \frac{h c}{\lambda} \Phi_0 (\lambda) d\lambda$  Eq (1-12)

Where $\Phi_0 (\lambda)$ is expressed as photons/time/area/bandwidth, here $h$ is the Planck’s constant and $c$ is the speed of light.

The electrical power output $P_{OUT}$ per area produced by the cell is given as the product of the current $I$ as a result of the incoming solar power times $V$ divided by the cell area given as

$P_{OUT} = JV$  Eq (1-13)

A plot of the possible $J-V$ operating points (called the light $J-V$ characteristics of the cell as shown in fig 1-15). The points $J_{sc}$ and $V_{oc}$ represent the extreme cases of the no voltage produced between the anode and the cathode (i.e., the illuminated solar cell is short-circuited) and of no current flowing between the anode and the cathode (i.e., the illuminated solar cell is open-circuited)
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Fig 1-12: The current density-voltage J-V characteristics of the photovoltaic cell under illumination.

The maximum power point (largest J-V product is also shown)

Device efficiency $\eta$ is defined as

$$\eta = \frac{J_{mp}V_{mp}}{P_{IN}} \text{Eq (1-14)}$$

Where, $P_{IN}$ is the incoming power per area. Ideally shaped J-V characteristics would be rectangular.

Fill factor is the measure of how close a given characteristic is to conforming to the ideal rectangular J-V shape. [76]

Fill factor $FF$ is given as

$$FF = \frac{J_{mp}V_{mp}}{J_{sc}V_{oc}} \text{Eq (1-15)}$$

Where, $FF \leq 1$.

1.2.5.b CYCLIC VOLTAMMETRY OF ELECTRODES

Cyclic voltammetry is a type of electro analytical technique to obtain information about the redox potential and electrochemical reaction rates of analyte
solutions. It is often used to characterize the performance of the electrochemical biosensors.

In cyclic voltammetry the voltage is switched between two values (V\(_2\) and V\(_1\)) at a fixed rate until it reaches the set potential (V\(_2\)). After reaching the set potential (V\(_2\)) the scan is reversed and the voltage is switched back to V\(_1\). The inversion of scanning can happen multiple times during a single experiment. The scan rate is a critical factor, since the duration of scan should provide sufficient time to allow the chemical reaction to be complete. Therefore varied results are obtained by varying the scan rate.

In this method the voltage is measured between the working electrode and counter-electrode. The current is plotted versus the voltage to obtain a voltammograms, which is used to study the electrochemical properties of the analyte in solution. The shape of the voltammograms for a specific compound depends on many factors such as scan rate, electrode surface and catalyst concentration [73].

1.2.5.c IMMUNOFLOURESCENCE TECHNIQUE

The combination of antibody with its specific antigen does not lead to a visible change and therefore a readily identifiable label must be irreversibly bound to the antibody so that its localization can be recognized. In immunoflourescence the label takes the form of a fluorochrome for example Flourescein or rhodamine which has the property of absorbing radiation in the form of ultraviolet or visible light. This absorbed radiation causes the molecule to attain an “excited state” leading to electron redistribution and the emission of radiation of a different wavelength. The emitted light is almost invariably of a longer wavelength and within the visible spectrum. In addition, the total emitted energy is less than that originally applied in the form of excitation energy.
There are mainly two types of immunoflourescence techniques – direct and indirect technique. Direct technique where the antibody conjugated with fluorochrome is used to detect the antigen when examined using ultraviolet light microscopy. Sandwich or indirect technique is the interaction between the antigen and antibody to which another antibody with fluorochrome conjugated is applied. Fluorescence indicated a reaction between the antigen and the antibody.

This method has the potential to define antigen-antibody interactions at the subcellular level such as detection of antibodies against mitochondria, microsomes, and smooth muscle fibers as well as identifying small cell-surface structures such as receptors on lymphocytes. [78]

This technique is used for single color imaging. This technique provides more room for detection of multiple markers, but quantitative analysis is compromised by the photo bleaching problem of organic fluorophore and the significant interference from tissue auto fluorescence. [79]

<table>
<thead>
<tr>
<th>Fluorochrome</th>
<th>Absorption maximum (nm)</th>
<th>Emission maximum (nm)</th>
<th>Observed colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flourescein (FITC)</td>
<td>494</td>
<td>518</td>
<td>green</td>
</tr>
<tr>
<td>Rhodamine (TRITC)</td>
<td>550</td>
<td>580</td>
<td>Red</td>
</tr>
<tr>
<td>Texas Red&lt;sup&gt;TM&lt;/sup&gt;</td>
<td>595</td>
<td>615</td>
<td>red</td>
</tr>
<tr>
<td>R-Phycoerythrin (PE)</td>
<td>565</td>
<td>575</td>
<td>Orange/red</td>
</tr>
</tbody>
</table>

Table 1-1: Table of different fluorochromes used
1.2.6 APPLICATIONS OF NANOPARTICLES

Nanomaterials have found a wide range of application from the field of engineering to bioengineering, computers to stain resistant cloths and suntan creams. Nanomaterial applications have been grouped into categories of catalysts, materials for energy conversion and storage and sensor based nanomaterials. [12] High surface area, selectivity features etc are helpful in making nanomaterials a well know candidate as catalysts for example gold-platinum nanocatalysts used in Li-air and Zn-air batteries.[80] In the nanosized alloy catalyst the platinum part is responsible for the reduction reaction and the gold part helps in oxidation.

Energy harvesting has involved in macroscopic equipment but in many nanomaterials is involved. Two most relevant fields are thermo- and piezoelectric conversion and solar energy conversion by solar cells. Thermoelectric nanomaterials convert thermal energy between two locations at different temperatures. The measure of the efficiency of a material in thermoelectric conversion is the ZT parameter, the ratio of Seebeck coefficient and the thermal and electrical conductivity of the material. Super lattices are the most efficient conversion devices but their fabrication cost is very high. The use of quantum dots in solar cells is the other way to apply nanomaterials in energy conversion in order to trap more photons or capture more energy from photons.

To achieve high sensitivity the material needs to have high dispersion, as surface atoms interact and undergo changes whereas atoms inside the material do not change meaning the higher the surface atom ratio, the larger the relative change in the measured physical property of the material. In sensors not only the size of the particle is important but also their shape. As the shape, number and configuration of edge and corner atoms change, further differences occur in the signals measured. Fluctuation based sensing or fluctuation enhanced sensing (FES) provides selectivity via investigation of fluctuation in resistance, voltage or current. Carbon nanotubes are especially useful and important building blocks of
FES devices, as they can be functionalized with appropriate chemical groups that make them selective for particular materials. [81]

![Applications of Nanoparticles](image)

Fig 1-14: Application of nanoparticles in a nut shell. [82]

1.3. SOLAR CELLS

A solar cell uses light absorbing materials that generate what is called an electron-hole pair when the material is illuminated. The process is called “excitation of charge carriers by light”. These carriers are separated in order to produce an external current through the load, resulting in electricity. The power output for all the quantum solar converters, including solar cell is the product of the flow of the photo-induced product and the driving force for the flow. For a solar cell, the flow is the electrical current, and
the driving force for the flow is related to the voltage. The voltage produced is dependent on the materials used. [83]

1.3.1 SILICON SOLAR CELLS

Silicon-based solar cells can be divided into two main groups: homojunction wafer based crystalline silicon c-Si solar cells and thin film silicon solar cells. Wafer based c-Si solar cells dominated the PV market in 2008 but the main challenge for these type of solar cells is the use of expensive wafers and the requirement of high temperature processing during junction formation.

Thin film silicon solar cells based on hydrogenated amorphous silicon (a-Si:H) and hydrogenated microcrystalline silicon (μc-Si:H) are promising candidates for low cost PV technology due to their low material consumption and low temperature processing in comparison to wafer based c-Si solar cells. Moreover, thin film silicon solar cells can be fabricated on a range of substrates, including flexible metal foils. However, low module efficiency of around 6 to 9 % is the principle limitation for this PV technology, which is a result of the poor electronic properties of the absorbers, such as a low carrier lifetime. The heterojunction silicon solar cell approaches benefit from combining both wafer-based c-Si and thin-film silicon solar cells. Not only does it improves the efficiency but also results in lower cost in comparison with c-Si solar cells. [84]
1.3.2 DYE SENSITIZED SOLAR CELLS

A DSSC is basically a thin layer solar cell formed by sandwich arrangements of two conducting oxides (TCO) electrodes. The main highly colored electrode has a few-micron-thick mesoporous TiO\textsubscript{2} layer coated with a photosensitizer. The counter-electrode is composed of islands of finely divided Pt deposited onto another TCO. The inter-layer space is filled with an organic electrolyte containing a redox mediator, usually a mixture of iodine and iodide in a low viscosity organic solvent such as acetonitrile. Best solar conversion efficiency obtained for this type of DSC is in the range of 11-12 % for laboratory scale cells (area < 1 cm\textsuperscript{2}) and around 8.5 % for large-area modules (100 cm\textsuperscript{2}). [85]
1.3.2.a  **SEQUENCE OF ELECTRON-TRANSFER STEPS OF A DSSC**

The absorption of light by the dye S leads to formation of its electronically excited state $S^*$.  

$$S + h\nu \rightarrow S^* \text{ (photoexcitation)} \quad \text{Eq (1-16)}$$

The molecule in the excited state can decay back to the ground state or undergo oxidative quenching, injecting electrons into the conduction band of TiO$_2$

$$S^* \rightarrow S + h\nu' \text{ (emission)} \quad \text{Eq (1-17)}$$

$$S^* \rightarrow S^+ + e^- \text{ TiO}_2 \text{ (charge injection)} \quad \text{Eq (1-18)}$$

The injected electrons travel through the mesoporous network of particles to reach the back-collector electrode to pass through the external circuit. The oxidised dye is reduced to the ground state by the donor (iodide) present in the electrolyte:

$$2S^+ + 3I^- \rightarrow 2S + I_3^- \text{ (regeneration of S)} \quad \text{Eq (1-19)}$$

In the absence of redox mediator to intercept and rapidly reduce the oxidised dye ($S^+$), recombination with the electrons of the Titania layer takes place, without any measurable photocurrent:

$$S^+ + e^- \text{ TiO}_2 \rightarrow S \text{ (recombination)} \quad \text{Eq (1-20)}$$
Fig 1-17: Schematic of DSSC showing principles of operation

The electrons reaching the counter-electrode through the external circuit reduce in turn the oxidised iodide (I) so that the entire sequence of the electron transfer reactions involving the dye and the redox mediator (I₂- I) is rendered cyclic:

\[ \text{I}^3^- + 2e^- \rightarrow 3 \text{I}^- \text{ (regeneration of I)} \]  \hspace{1cm} \text{Eq (1-21)}

If these reactions alone take place, then the overall effect of irradiation with sunlight is to drive the electrons through the external circuit, i.e., direct conversion of sunlight to electricity.

1.3.2.b  KEY PARAMETERS OF DSSC

Characterization of the cell depends on a number of experimentally accessible parameters including the photocurrent and photopotentials measured under different conditions (open and closed circuit, under monochromatic light or sunlight illumination): \( I_{oc}, V_{oc}, I_{sc} \) and \( V_{sc} \). The term incident photon-to electrical conversion efficiency (IPCE) is a quantum yield term for the overall charge-injection collection process measured using monochromatic light (single wavelength source).

\[ \text{IPCE} (\lambda) = 1240 \left( \frac{I_{sc}}{\lambda_0} \right) \]  \hspace{1cm} \text{Eq (1-22)}
Where $\lambda$ is the wavelength, $I_{sc}$ the current at short circuit ($\text{mA/cm}^2$) and $\varphi$ is the incident radiation flux ($\text{W/m}^2$)

![Graph](image)

Fig 1-18:(a): The monochromatic wavelength dependence of photocurrent and Fig (b) the photocurrent-voltage curve of a solar cell based on Ru-bpy sensitizer N945 measured under AM 1.5 illumination (100 mWcm$^{-2}$)

The overall sunlight to electric power conversion efficiency of a DSSC is given by

$$\eta = \frac{P_{\text{max}}}{P_{\text{in}}} = \frac{I_{sc}V_{oc}FF}{P_{\text{in}}} \quad \text{Eq (1-23)}$$

The overall sunlight-to-electric-power conversion efficiency of a DSSC can be expressed as the product of three key terms

$$H = \eta_{\text{abs}} \cdot \eta_{\text{inj}} \cdot \eta_{\text{coll}} \quad \text{Eq (1-24)}$$

Where $\eta_{\text{abs}}$ is the efficiency of light absorption by the dye

$\eta_{\text{inj}}$ is the efficiency of the charge injection from the excited state of the dye

$\eta_{\text{coll}}$ is the efficiency of charge collection in the mesoporous oxide layer.

An ideal photosensitiser will absorb all the sunlight in the visible-near IR region with high absorption cross section (coefficient).

The key components of DSSC include the substrate materials such as mesoporous TiO$_2$ layer, substrate glass with transparent conducting oxide TCO layer, dye electrolyte solvent, redox mediator and a counter electrode. [85]
1.3.2.c DSSC USING NATURAL DYES:

Although high performance synthetic ruthenium-free dyes have been developed the synthesis is time-consuming and laborious. Their tests for toxicity need to be done before they are used commercially. Therefore natural dyes are used since these are easily and safely extracted from plants which means they are cheap and widely available and do not require complex synthesis or toxicity testing. Natural chlorophyll dyes achieved energy conversion efficiencies of over 4% [86-87]. Most of the other dyes tested yielded energy conversion efficiencies below 2%. [88-90]

<table>
<thead>
<tr>
<th>Plant source</th>
<th>Structure or structural class</th>
<th>Photovoltaic current density ($I_{sc}$) (mA/cm²)</th>
<th>$V_{oc}$ (mV)</th>
<th>$\eta$ (%)</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta vulgaris rubra</td>
<td>Betaines</td>
<td>0.5</td>
<td>0.5</td>
<td>425</td>
<td>1.70/0.77</td>
</tr>
<tr>
<td>Rhoeas sphaerica finden</td>
<td>-</td>
<td>1.60</td>
<td>406</td>
<td>15.0/77</td>
<td>Aquous extract, aqueous electrolyte</td>
</tr>
<tr>
<td>Bixa orizana L.</td>
<td>Bixin</td>
<td>0.5</td>
<td>1.1</td>
<td>570</td>
<td>0.37/0.59</td>
</tr>
<tr>
<td>Spinach</td>
<td>Modified chlorophyll/aesculina</td>
<td>11.8</td>
<td>550</td>
<td>3.0/4.0</td>
<td>Isolated compounds</td>
</tr>
<tr>
<td>Spinach</td>
<td>Modified chlorophyll/daucus</td>
<td>-</td>
<td>11.4</td>
<td>540</td>
<td>3.0/4.0</td>
</tr>
<tr>
<td>Spinach</td>
<td>Modified chlorophyll/kainia</td>
<td>-</td>
<td>2.5</td>
<td>740</td>
<td>3.0/4.0</td>
</tr>
<tr>
<td>Spinach</td>
<td>Modified chlorophyll/cis-iso-cis</td>
<td>-</td>
<td>1.60</td>
<td>390</td>
<td>0.48</td>
</tr>
<tr>
<td>Cabbage-palm fruit</td>
<td>-</td>
<td>0.86</td>
<td>422</td>
<td>0.43</td>
<td>Extract</td>
</tr>
<tr>
<td>Celatine fruit</td>
<td>Diphosphin</td>
<td>-</td>
<td>0.86</td>
<td>520</td>
<td>0.50</td>
</tr>
<tr>
<td>Jasminum sinense</td>
<td>Jasmin</td>
<td>-</td>
<td>2.6</td>
<td>650</td>
<td>0.62</td>
</tr>
<tr>
<td>Gardenia fruit</td>
<td>Crocin/</td>
<td>5.0</td>
<td>2.84</td>
<td>430</td>
<td>0.50/0.46</td>
</tr>
<tr>
<td>Red Sirakan orange “Meta”</td>
<td>Gymn</td>
<td>0.5-1.0</td>
<td>3.84</td>
<td>540</td>
<td>0.66/0.50</td>
</tr>
</tbody>
</table>

| Table 1-2: Table of natural dyes used in DSSC with their corresponding photocurrent efficiencies [90] |
The photovoltaic cell’s efficiency does not only depend on the pigment’s molecular structure, but also strongly on solid-state properties, such as morphology, self-assembly and aggregation of the dye molecules. In this case the DSSC even more parameters have an influence on the efficiency such as the type of photo electrode; the dye’s anchoring group and the electrolyte with the respective redox couple. The design of a photosensitizer consists of a donor-acceptor- substituted π-conjugated bridge fig (1-20). The acceptor part is the dye’s anchoring group which is attached to the TiO$_2$.

![Donor-π-acceptor structure principle of an organic dye in DSSC with TiO$_2$ photo-anodes](image)

In natural dyes the functional groups necessary to interact with the TiO$_2$ surface is a carboxylic or other peripheral acidic anchoring group. [91-92] In order to form bonds, the dye’s binding groups react with the surface hydroxyl groups of the metal oxide. In case of phosphonic acid or carboxylic acids, a reversible binding with high equilibrium binding constants is established between the photosensitizer and TiO$_2$. Out of the various binding modes possible which mode of the chemisorption between TiO$_2$ and -COOH is prevalent depends on the dye’s structure, its anchoring groups and the metal oxide preparations. [92]
1.4. BIOSENSORS

The accurate analyses of biological materials are expensive and need to be performed in external laboratories equipped with more sophisticated instrumentation. Most of these analyses require previous purification that requires too much time relative to the processing time, making their on-line implementation impossible for control purpose. However in living organisms, biological components like antibodies and enzymes work as natural sensing and controlling “devices”. The ability of isolating and purifying these proteins and other biological elements such as cells or organelles has allowed their integration with physicochemical transduction devices to produce biosensors.

It is defined as

“A self-contained analytical device that incorporates a biologically active material in intimate contact with an appropriate transduction element for the purpose of detecting (reversibly and selectively) the concentration or activity of chemical species in any type of sample.”[93]

Fig 1-21: General principle of a biosensor [98]

The first biosensor was an enzyme-based glucose sensor which was developed by Clark and Lyons [94].
The basic characteristics of a biosensor are

(1) Linearity

(2) Sensitivity

(3) Selectivity

(4) Response time

1.4.1 TYPES OF BIOSENSORS

Biosensors can be classified based on their biological element or their transduction element. Biological elements include enzymes, antibodies, micro-organisms, biological tissue, and organelles. [95]

When the binding of the sensing element and the analyte is the detected event, the instrument is described as an **affinity sensor**.

When the interaction between the biological element and the analyte is accompanied or followed by a chemical change in which the concentration of one of the substrates or products is measured the instrument is described as a **metabolism sensor**.

When the signal is produced after binding the analyte without chemically changing it but by converting an auxiliary substrate the biosensor is called **catalytic sensor**. [96]

Another classification of biosensor is based on the transduction modes used. On the basis of different transducers biosensors can be electrochemical (amperometric, conductometric, and potentiometric), optical (absorbance, fluorescence, chemiluminescence), piezoelectric (acoustic and ultrasonic) and calorimetric. [95]

**In Amperometric biosensor**, the potential between two electrodes is set and current produced by the oxidation or reduction of electro active species is measured and correlated to the concentration of the analyte eg, glucose biosensors for diabetes monitoring.[97]
Potentiometric biosensor uses ion selective electrodes to determine changes in the concentration of chosen ions e.g., use of H+ ions for penicillin detection using enzyme penicillinase, triacyl glycerol using lipase. [97]

Optical biosensor is in which Fiber active probes are used and on the tip of which enzymes and dyes (often fluorescent) are coated. Light interacts with the reagents are placed near the tip of the optical fiber, after interaction light returns with an intensity which is measured and indicates the amount of analyte eg optic lactate sensor using lactate monooxygenase and oxygen.[97]

Calorimetric biosensor - Many enzymes catalysed reaction are exothermic generating heat which is used as a basis for measurement of rate of reaction and hence analyte concentration. The temperature changes are determined by thermistors eg. Cholesterol biosensors using cholesterol oxidase (heat output 53 KJ mol⁻¹). [97]

1.4.2 IMMUNOSENSORS

Anti-bodies based sensors are called immunosensor. Antibodies frequently have been used for the detection of various analytes due to their high specificity. Antibodies may be immobilized on for example, optical fibres, electrodes or semiconductor chips. [99-101] Different immobilization chemistries and strategies have been utilised. [102] Linkages to solid surfaces are frequently made by glutaraldehyde, carbodiimide and other reagents such as succinimide ester, maleimide and periodate. Care is to be taken during the immobilization procedure that the specificity of the biosensors is not lost. Some factors that decrease the specificity of biosensors include the cross-reactivity of enzymes, nonspecific binding (analyte binding at the place where it should not) and interferences at the transducer [103].
The figure 1-22 shows the schematic diagram of an antibody molecule with its fragments [103]. The Fc fragment comprises the effector functions such as complement activation, cell membrane receptor interaction and transplacental transfer [104]. The F(ab’)2 contains two identical Fab’ fragments, which are held together by disulfide linkages in the hinge (H) region. The Fab’ fragments contain the antigen-binding site. The VH and VL are the variable heavy and light chains, respectively. The CH1 and CH2 are the constant chains. Note that as the antibody is immobilized on a support it generally loses some of its activity as shown in fig 1-23 where in some orientations (or conformations) inhibit the formation of the antigen-antibody complex. The ability of the antibody to bind with antigen in liquid may be totally lost when on solid substrates. Therefore one needs to be very careful to preserve the inherent antibody activity. [102] Thus several approaches have been developed to obtain an appropriate orientation of the antibody during the immobilization process as shown in fig 1-24.
Fig 1-23: IgG antibody configurations on a surface during a random coupling procedure [102]

Fig 1-24: Oriented antibody immobilization [102] (a) antibody binds to the Fc receptors on surface (b) antibody is bound to a solid support through an oxidised carbohydrate moiety on its C_H2 domain of the Fc fragment (c) monovalent Fab’ fragment bound to insoluble support through a sulfhydryl group in the C-terminal region.

Compared to a chemical sensor the antigen antibody binding interactions are far specific in nature. Also the detections can be carried out at very low concentration as shown in the fig 1-25.
1.4.2.a ASSAY FORMATS

Four typical assay formats are used [106]

(a) Direct binding - The analyte in the solution binds directly to the receptor on the surface, and the analyte-receptor complex is monitored. For example, if an antigen in solution binds to the antibody immobilized on the biosensor surface, it is an immunoassay format.

(b) Sandwich assay - When low molecular weight antigens (hapten, Ag) are to be analysed, one often resorts to sandwich assays. This is because the binding of the hapten to primary antibody (Ab₁) in this case induces only insignificant or very small changes in the signal to be measure. A secondary antibody (Ab₂) is simultaneously added to the analyte (antigen) in solution or soon thereafter a complex formed (Ab₂. Ab₁. Ag) is continuously monitored.
(c) Displacement assays – Analyte to be measured is immobilized on the biosensor surface. Then the corresponding antibodies bound. On addition of the analyte in solution to be measured, antibodies are displaced from the surface and bind to the free antigens. In this case one observes and measures a negative change.

(d) Replacement assays - The surface is coated with the antibody molecules. An analogue of the antigen (conjugated to larger molecule) is bound to the antibody immobilized. This is the refractive index probe. [108] On the addition of the analyte in the solution to be measured to the biosensor, the analyte partially replaces the bound conjugated antigen and a negative index change is measured.

Fig 1-26: Different forms of immunoassays. Immobilized molecules shown at left; the addition of analyte in the middle; resulting ad layer on the surface on the right in (a) Direct binding; (b) sandwich assay; (c) displacement assay; (d) replacement assay.[107]
1.4.2.b  DIRECT AND INDIRECT IMMUNOSENSORS

If antigen-antibody binding takes place on the transducer surface, the properties of the solid phase are altered. The increase in layer thickness or surface coverage can be measured in terms of the change in refractive index, mass increase or reflectivity properties. A device capable of determining any of these parameters directly associated with the immunoreactions on the surface is referred to as direct immunosensor.

Another approach is signal generation by tagging one of the complementary reaction partners (e.g. The antigen with some label capable of signal generation). A typical label common in immunoassays would be a radioactive marker, an enzyme, an electro active compound or a fluorophore. The use of label yields an indirect immunosensor whose signal is produced by a secondary compound or reaction. In cases where the analyte itself is fluorescent properties no label is required, thus yielding a sensor of the direct type although measuring fluorescence.

The problem of nonspecific binding is more in direct immunosensor where as in indirect immunoassays this problem is partly overcome by sample dilution and addition of detergents and other reagents which reduces the hydrophobic interactions. [98]

1.5. OBJECTIVES OF PRESENT STUDY

The general objective of the dissertation study focuses on investigating the novel and facile synthesis routes of various Nanomaterials ranging both 1-dimensional and 2-dimensional nanostructures and their potential applications to immunosensing and solar cell applications. A substantial understanding of the interactions of graphene and various Nanomaterials with biomolecules enables to improve the applications of the various nanostructures in biosensor as well as photovoltaic devices. Novel synthesis of reduced graphene oxide promotes its application as transparent electrode in photovoltaic cell. Studies of reduced graphene oxide and heterogeneous graphene-Cu nanostructures promote an understanding of the interface between
carbon and inorganic and helps in applying reduced graphene oxide for immobilization of antibodies thereby acting as a unique platform for immuno sensing.

To achieve the general objective the following particular ones were established.

Objective 1: Identification of natural pigment, chlorophyll, as the candidate dye for dye sensitized solar cell and synthesis of nanomaterial for the efficient sensitization of the dye. Chlorophyll dye, universally accepted as a natural dye for photo energy conversion occurs naturally as Chl-a and Chl-b forms. The huge bio molecules are highly water repelling partly because of the long phytol chain in the molecule. The organic synthesis of the molecule is highly expensive because each ring contains 4 nitrogen atoms. It is also seen that Chl-a traps light energy but Chl-b differs from Chl-a by having a –CHO group in place of –CH$_3$ group in the latter making the binding relatively difficult. Due to these reasons, the overall dye sensitization is reduced and hence the efficiency of the dye sensitized solar cells so developed is also reduced.

1.1. For improving the overall sensitization, various metal oxide nanomaterials were synthesized using modified precipitation synthesis method.

1.2. Characterization of the nanoparticles using various techniques such as XRD, SEM, TEM, etc.

1.3. Use of the nanoparticles as sensitizer for the dye in dye sensitized solar cells

Objective 2: Synthesis of reduced graphene oxide by a novel route.

A novel route of synthesis of reduced graphene oxide is being proposed. The conventional method proposed by Hummer et al used acid treatment of graphite for oxidation. The novel route neither uses any acid-base reagent for the synthesis but solely relies on a particular combination of alcohol-ketone-surfactant which under mechanical and low temperature treatment results in the oxidation of graphite. The material characterization is more studied.
Chapter 1 General Introduction

2.1. Synthesis of reduced graphene oxide by a novel route

2.2. Characterization of the material by various techniques – optical, electrical, morphological etc.

Objective 3: Application of reduced graphene oxide synthesized by novel route in dye sensitized solar cells counter electrodes instead of aluminum or silver electrode.

Transparent as well as conducting counter electrodes are better preferred for the development of a dye sensitized solar cell. Reduced graphene oxide being transparent and highly conducting was being studied. Further the problem of corrosion is totally overcome by the use of reduced graphene oxide.


3.2. Study of performance of dye sensitized solar cells using reduced graphene oxide counter electrode.

Objective 4: Study of the application of reduced graphene oxide synthesized by the novel route as the basic platform for the immobilization of antibodies.

Sensitive and accurate detection of disease related proteins is a fundamental requirement for clinical diagnostics. The structure, conductivity, surface area and biocompatibility of the electrode interface play a central role on the electron transfer, mass transport and biomolecules activity. In addition to the electronic features of reduced graphene oxide the presence of small quantities of functional groups such as hydroxyl, carboxyl and epoxy causing a negative charge on the material makes it to be an ideal platform for building sensing devices.

4.2. Study of confirmation of Immobilization of antibodies by the AFM, SEM, Four probe measurements- conductivity studies, cyclic voltammetry etc.

**Objective 5: One step synthesis of graphene nano sheets decorated with metal nanoparticles for immobilization of antibodies.**

The immobilization of biomolecules is crucial for developing efficient immunosensor. Graphene with its low electrical resistance, atomic thickness and two dimensional structures provides a large specific surface area for the immobilization. The electron transport through graphene can be highly sensitive to the absorbed molecules. By modifying graphene with various noble metals and adjusting the number of layers various characteristics and tuned catering to the need of maximum immobilization of the antibodies.

5.1. Synthesis of various metal decorated graphene Nanosheets were done using one step facile synthesis method.

5.2. Characterization of the nanomaterial using various techniques such as SEM, XPS, AFM, TEM etc. were done.

5.3. Antibody immobilization was confirmed by the AFM, SEM, Cyclic voltammetry etc.

**Objective 6: Investigating the secondary binding of anti-species to the antibody immobilized on the substrate material.**

Antibody- antigen is a specific type of binding interaction. Antibody also interacts with its anti-species falls into a new approach of studying the immobilization of antibodies and therefore aids to studies of the functioning of the electrode for an immune sensor.
Chapter 1 General Introduction

6.1. To evaluate the specificity of the immunoassay by using fluorophore tagged anti-species.

6.2. To evaluate the performance of the immune sensor by defining its operational range, detection limit, etc