MODULE 6

MODERN TECHNIQUES FOR MATERIAL STUDIES, INTRODUCTION TO BIOMATERIALS AND NANOMATERIALS

MODERN TECHNIQUES FOR MATERIAL STUDIES



- *Microscope* is the combination of two words; "micro" meaning small and "scope" meaning view.
- Resolution The amount of detail you can see in an image.
- The **optical microscope**, often referred to as **light microscope**, is a type of microscope which uses visible light and a system of lenses to magnify images of small samples.
- Basic optical microscopes can be very simple, although there are many complex designs which aim to improve resolution and sample contrast.
- Light microscopes use lenses and light to magnify cell parts.
- There are two basic types of optical microscopes:
 - Simple microscope
 - \circ Compound microscope

Advantages:

- •Cheap to purchase and operate
- •Small and portable
- •Natural colour of the specimen can be observed
- •Living as well as dead materials can be viewed
- •Preparation is relatively quick and simple
- •Unaffected by magnetic fields

- •Magnification is low
- •Preparation may distort specimen
- •Limited resolution and poorer surface view of the specimen.
- •The depth of the field is restricted

\circ Simple microscope

- A simple microscope is one which uses a single lens for magnification, such as a magnifying glass.
- It's total magnification is limited to the magnification of the single lens used.
- Condenser lens is absent
- Light source is natural
- Stand is small, hollow cylindrical attached to the base and is used to hold the microscope.
- Mirror is concave-reflecting type.
- Has only one adjustment screw that is used to move the limb up and down for focusing an object.
- Can only be used in simple ways such as enlarging small letters while reading.

\circ Compound microscope

- Has two sets of lenses for magnifying objects: eyepiece lens and objective lenses
- Its total magnification is the multiplication of the eyepiece and objective magnifications, hence has a higher magnification.
- Condenser lens is present which is used to adjust the intensity of light for magnification of object.
- Illuminator is a source of light which is helpful when small, minutest pieces needed to be seen.
- Mirror is plane at one side and concave at other side
- Has coarse adjustment screw (for rapid focusing an object) and fine adjustment screw (for fine and sharp focusing).
- Has a wide range of use such as in studying the structure of different objects, e.g. details of cells in living organisms.



- The major components of light microscope are;
 - \circ Ocular lens or eyepiece is used for viewing.
 - Revolving nosepiece contains objective lenses that are used to magnify the image in combination with the ocular lens.
 - $\circ~$ Stage- the location of the specimen to be viewed
 - $\circ~$ Clips- utilized in holding the specimen in place
 - Lamp- typically a light source underneath the stage
 - Diaphragm- controls the amount of light allowed to pass through the specimen
 - Course adjustment knob- is the larger of the two knobs used to bring the object into quick focus.
 - Fine adjustment knob- is used for improving the clarity of the image, especially when viewing under high power.



Electron Microscope:

- A special type of microscope having a high resolution of images
- It can magnify objects in nanometers, which is possible by controlled use of electrons in vacuum and captured on a phosphorescent screen.
- The novelty of EMs from others;
 - Beam of Electrons instead of a beam of light
 - $\circ~$ Electro-magnetic lens instead of Ground glass lenses
 - Cylindrical Vacuum column Electrons should travel in vacuum to avoid collisions with air molecules that cause scattering of electrons distorting the image
- Types of electron microscope;
 - $\circ\,$ Transmission Electron Microscopy (TEM)
 - \circ Scanning Electron Microscopy (SEM)

✤ <u>Transmission Electron Microscope (TEM)</u>:

- A light source at the top of the microscope emits the electrons that travel through vacuum in the column of the microscope.
- Instead of glass lenses focusing the light in the light microscope, the TEM uses electromagnetic lenses to focus the electrons into a very thin beam.
- The electron beam then travels through the specimen you want to study. Depending on the density of the material present, some of the electrons are scattered and disappear from the beam.
- At the bottom of the microscope the electrons not scattered hit a fluorescent screen, which gives rise to a shadow image of the specimen with its different parts displayed in varied darkness according to their density.
- The image can be studied directly by the operator or photographed with a camera.

Transmission Electron Microscope Working:

- The electron beam from the electron gun can be focused and defocused by a series of electromagnetic lenses.
- The Condenser Lenses concentrate the beam onto the specimen.
- Electrons passing through the specimen will be focused by the Objective & Intermediate lenses to form an intermediate image.
- The Projector lens enlarges this image into a final image on the fluorescent viewing screen at the bottom of the microscope column.
- Each lens is basically a circular electro-magnet.
- A variable electric current through the lens will produce a magnetic field of variable strengths which will deflect or bend the electron beam passing through



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Scanning Electron Microscope (SEM):

- The SEM uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens.
- Electrons are produced at the top of the column, accelerated down and passed through a combination of lenses and apertures to produce a focused beam of electrons which hits the surface of the sample.
- The sample is mounted on a stage in the chamber area and, unless the microscope is designed to operate at low vacuums, both the column and the chamber are evacuated by a combination of pumps.
- The level of the vacuum will depend on the design of the microscope
- The position of the electron beam on the sample is controlled by scan coils situated above the objective lens.

Scanning Electron Microscope (SEM):

- The position of the electron beam on the sample is controlled by scan coils situated above the objective lens.
- These coils allow the beam to be scanned over the surface of the sample.
- This scanning beam, enables information about a defined area on the sample to be collected.
- As a result of the electron-sample interaction, a number of signals are produced. These signals are then detected by appropriate detectors.



Comparison Transmission Scanning Light Microscopy Electron Microscopy Electron Microscopy Light Source Electron Source (Lamp) Condenser Condenser Lens Lens Scanning Coil Specimen (Beam Deflector) (Tissue Sections) Objective Condenser Lens Lens Detector Eyepiece Projection Lens Lens Electrons 3-Dimensional Specimen Image Viewed Image Viewed on Image Viewed Fluorescent Screen on Monitor Directly

- Photoemission spectroscopy (PES), also known as photoelectron spectroscopy, refers to energy measurement of electrons emitted from solids, gases or liquids by the photoelectric effect, in order to determine the binding energies of electrons in a substance.
- Photoelectron spectroscopy uses the phenomenon of photo electric effect to learn about the electronic structure of matter
- It refers to the various techniques, depending on whether the ionization energy is provided by an X ray photon, an EUV photon, or an ultraviolet photon.
- Regardless of the incident photon beam, all photo electron spectroscopy revolves around the general theme of surface analysis by measuring the ejected electrons.

Types of photo electron spectroscopy:

- X ray Photo electron Spectroscopy
 - X-ray Photoelectron Spectroscopy (XPS), also known as Electron Spectroscopy for Chemical Analysis (ESCA) is a widely used technique to investigate the chemical composition of surfaces (usually solids).
 - X-ray Photoelectron spectroscopy, based on the photoelectric effect, was developed in the mid- 1960's by Kai Siegbahn and his research group at the University of Uppsala.



X – ray Photo electron Spectroscopy

- X-ray Photoelectron Spectroscopy (XPS) requires ultra high vacuum. UHV removes absorbed gases from the sample, it eliminates absorption of contaminants on the sample, it prevent arcing and high voltage breakdown, also, it increases mean free path electrons, ions and photons.
- Applications:
 - ✓ Measurement of 1. Elemental composition
 - 2. Empirical formula determination
 - 3. Chemical state
 - 4. Electronic state
 - 5. Binding energy
 - 6. Layer thickness in the upper portion of surfaces

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- \checkmark Analysis of stains and residues on surfaces
- ✓ Imaging of surfaces

X – ray Photo electron Spectroscopy

• Limitations:

- \checkmark The smallest analytical area XPS can measure is ~10 μm
- ✓ Samples for XPS must be compatible with the ultra high vacuum environment
- ✓ XPS is limited to measurements of elements having atomic numbers of 3 or greater, making it unable to detect hydrogen or helium.
- \checkmark XPS spectra also take a long time to obtain.
- Advantages:
 - ✓ XPS is good for identifying all (except two) elements, identifying the chemical state on surfaces, and is good with quantitative analysis.
 - ✓ XPS is capable of detecting the difference in chemical state between samples.
 - ✓ XPS is also able to differentiate between oxidations states of molecules

- <u>Ultraviolet Photo electron Spectroscopy (UPS)</u>
 - \circ UV light (hv = 5 to 100 eV) is used to excite photoelectron.
 - In early UPS, the sample was a gas or a vapor that is irradiated with a narrow beam of UV radiation.
 - More modern UPS instruments are now capable of studying solids as well.
 - The photoelectrons produced are passed through a slit into a vacuum region where they are then deflected by magnetic or electrostatic fields to give an energy spectrum.
 - UPS is sensitive to the very near surface region, up to around 10 nm in depth.



<u>Ultraviolet Photo electron Spectroscopy (UPS)</u>

- $\circ~$ There are two main areas UPS is used to study:
 - \checkmark Electronic structure of solids
 - ✓ Adsorbed molecules on metals
- Limitations:
 - ✓ UPS is capable only of ionizing valence electrons, which limits the range and depth of UPS surface experiments.
 - \checkmark Conventional UPS has relatively poor resolution.
- Advantages:
 - ✓ Ultraviolet radiation has a very narrow line width and a high flux of photons available from simple discharge sources.
 - ✓ Higher resolution UPS scans allow the observation of fine structures due to vibrational levels of the molecular ions which, then, allows molecular orbital assignment of specific peaks.

ATOMIC ABSORPTION SPECTROSCOPY

- Atomic Absorption Spectroscopy (AAS) is a very common technique for detecting metals and metalloids in samples.
- It is very reliable and simple to use.
- It can analyze over 62 elements.
- Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.
- The first atomic absorption spectrometer was built by scientist Alan Walsh in 1954.

Principle:

- The technique is based on the principle that free atoms (gas) generated in an atomizer can absorb radiation at specific frequency.
- Atomic-absorption spectroscopy quantifies the absorption of ground state atoms in the gaseous state .
- The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The analyst concentration is determined from the amount[®] of absorption.

ATOMIC ABSORPTION SPECTROSCOPY

• Schematic diagram of Atomic Absorption Spectrometer



- Hollow Cathode Lamp are the most common radiation source in AAS.
- It contains a tungsten anode and a hollow cylindrical cathode made of the element to be determined.
- These are sealed in a glass tube filled with an inert gas (neon or argon)
- Each element has its own unique lamp which must be used for that analysis.
- A large voltage across anode and cathode causes the inert gas to ionize and form a plasma
- These ions are accelerated towards the cathode causing atoms to be sputtered.
- The ions and metal atoms are excited due to collisions.
- They give off photons of a certain wavelength when they reach ground state.



- The **nebulizer** forms a mist or aerosol of the sample
- This is done by forcing the sample at high velocities through a narrow tube
- The sample is mixed with a fuel and oxidant thoroughly for introduction into flame.
- Commonly used **fuel-oxidant mixtures** are acetylene-air and acetylene-nitrous oxide.
- Elements to be analyzed needs to be in atomic state. In the **atomizer** the sample solutions is vaporized and the molecules are atomized
- Atomization is separation of particles into individual molecules and breaking these molecules into atoms. This is done by exposing the analyst to high temperatures in a flame or graphite furnace.
- There are two type of atomizers; flame atomizers and graphite tube atomizers.

- Flame atomizer uses an air-acetylene or nitrous oxide acetylene flame to remove the solvent and bring the element in the sample to ground state.
- The ground state atoms absorb light from the hollow cathode lamp and produce signal proportional to the concentration of atoms in the light path
- Graphite tube atomizers use a graphite coated furnace to vaporize the sample.
- In GFAAS, samples are deposited in a small graphite coated tube which can then be heated to vaporize and atomize the analyst.
- \circ The graphite tubes are heated using a high current power supply.
- A monochromator is used to separate out the thousands of lines.
- It select the specific wavelength of light which is absorbed by the sample, exclude other wavelengths.
- The selection of the specific light allows the determination of the selected element in the presence of others.

- The light selected by the monochromator is directed onto a **detector** that is typically a photomultiplier tube, whose function is to convert the light signal into an electrical signal proportional to the light intensity.
- The processing of electrical signal is fulfilled by a **signal amplifier**. The signal could be displayed for readout, or further fed into a data station for printout by the requested format.
- A calibration curve is used to determine the unknown concentration of an element in a solution. The instrument is calibrated using several solutions of known concentrations. The absorbance of each known solution is measured and then a calibration curve of concentration vs absorbance is plotted.
- The sample solution is fed into the instrument, and the absorbance of the element in this solution is measured. The unknown concentration of the element is then calculated from the calibration curve

ATOMIC ABSORPTION SPECTROSCOPY

- Applications:
 - Determination of even small amounts of metals like lead, mercury, calcium, magnesium, etc..
 - Environmental studies: drinking water, ocean water, soil.
 - Food industry.
 - Pharmaceutical industry.
 - Determination of lead in petrol
 - Level of metals could be detected in tissue samples like Aluminum in blood and Copper in brain tissues
- Advantages
 - High sensitivity
 - Easy to use
 - Not that much expensive
- Disadvantages
 - Different cathode lamp for different elements
 - Can detect only metals and some non metals
 - Only one element detected

INTRODUCTION TO BIOMATERIALS AND NANOMATERIALS

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- Nano materials are commonly defined as materials with an average grain size less than 100 nanometres (One billion nanometers equals one meter)
- Nano materials can be
 - \circ **One dimension** (surface films)
 - **Two dimensions** (strands or fiber) Eg: nano wires & nano tubes
 - Three dimensions (particles) Eg: colloids, quantum dots
 - They can exist in single or fused forms with spherical, tubular, and irregular shapes.
- Small particles are 'invisible', hence, transparent coatings or films are attainable
- Small particles are very weight efficient enabling surfaces to be modified with minimal material

- Two principal factors that cause the properties of Nano materials to differ significantly from other materials are increased relative surface area and quantum effects.
- These factors can change or enhance properties such as reactivity, strength and electrical characteristics.
- As a particle decreases in size, a greater proportion of atoms are found at the surface compared to those inside.
- Thus Nano particles have a much greater surface area per unit mass compared with larger particles.
- Growth and catalytic chemical reactions occur at surfaces, hence, Nano particulate form will be much more reactive than the same mass of material made up of larger particles.
- By patterning matter on the Nano scale, it is possible to vary fundamental properties of materials without changing the chemical composition

Methods for creating Nano structures:

• Top-down

- \circ It involves breaking down matter into more basic building blocks.
- \circ Frequently uses chemical or thermal methods.

• Bottoms-up

- It involve the assembly of atoms or molecules into nanostructured arrays.
- In these methods the raw material sources can be in the form of gases, liquids or solids.

TYPES OF NANO MATERIALS

Different types of Nano materials:

- Natural Nano materials:
 - These include Nano materials that exist in biological systems
 - Eg.: viruses(capsid), substances in our bone matrix,..
- Artificial Nano materials:
 - These are the ones that are fabricated by different experiments.
 - \circ They can be further subdivided into four categories;
 - 1. Carbon based:
 - These nanomaterials are composed mostly of carbon, taking the form of a hollow spheres, ellipsoids, or tubes.
 - Spherical and ellipsoidal carbon Nano materials are referred to as **fullerenes**, while cylindrical ones are called nanotubes (**carbon nanotubes**, CNTs).



TYPES OF NANO MATERIALS

2. Metal Based:

• These Nano materials include quantum dots, Nano gold, Nano silver and metal oxides, such as titanium dioxide.

3. Dendrimers:

- These Nano materials are Nano sized polymers built from branched units.
- The surface of a dendrimer has numerous chain ends, which can be tailored to perform specific chemical functions. This property could also be useful for catalysis.

4. Composites:

- Composites combine Nano particles with other nanoparticles or with larger, bulk-type materials.
- The composites may be any combination of metal based, carbon based or polymer based Nano materials with any form of metal, ceramic, or polymer bulk materials

Properties:

- Due to their small dimensions, Nano materials have extremely large surface area to volume ratio, which gives it more surface dependent material properties.
- Optical properties of Nano materials gives various applications such as optical detector, sensor, imaging, display, solar cell, photo catalysis, photo electrochemistry and biomedicine
- Nano materials possess good mechanical, electrical and magnetic properties

- Instability of the particles
 - Nano materials possess poor corrosion resistance, high solubility, and phase change. This leads to deterioration in properties and retaining the structure becomes challenging.

- Fine metal particles act as strong explosives owing to their high surface area coming in direct contact with oxygen. Their exothermic combustion can easily cause explosion
- Because nanoparticles are highly reactive, they inherently interact with impurities as well.
- Biologically harmful
 - Nano materials are considered harmful as they become transparent to the cell dermis.
 - Toxicity appears predominant owing to their high surface area and enhanced surface activity.
 - Nano materials have shown to cause irritation, and are indicated to be carcinogenic.
 - If inhaled, their low mass entraps them inside lungs and in no way they can be expelled out of body.
 - \circ Their interaction with liver or blood could also prove to be harmful

- Difficulty in synthesis and isolation
 - It is extremely hard to retain the size of nanoparticles once they are synthesized in a solution. Hence, the Nano materials have to be encapsulated in a bigger and stable molecule.
 - Free nanoparticles are hard to be utilized in isolation, and they have to be interacted for intended use via secondary means of exposure.
- Recycling and disposal
 - $\circ~$ There are no safe disposal policies evolved for Nano materials.
 - Issues of their toxicity are still under question, and results of exposure experiments are not available.

• Fuel cells

(A fuel cell is an electrochemical energy conversion device that converts the chemical energy from fuel on the anode side and oxidant on the cathode side directly into electricity.)

- The performance of a fuel cell electrode can be optimized by improving the physical structure of electrode and by using more active electro catalyst.
- A good structure of electrode must provide ample surface area, provide maximum contact of catalyst, reactant gas and electrolyte, facilitate gas transport and provide good electronic conductance
- Carbon nanotubes (CNTs) have chemical stability, good mechanical properties and high surface area, making them ideal for the design of sensors and provide very high surface area due to its structural network.

- **Microbial fuel cell** is a device in which bacteria consume water-soluble waste such as sugar, starch and alcohols and produces electricity plus clean water.
 - It can turn different carbohydrates and complex substrates present in wastewaters into a source of electricity. The efficient electron transfer between microorganism and the anode of the microbial fuel cell plays a major role in the performance of the fuel cell
 - Carbon nanotubes are suitable to support cell growth. Hence, electrodes of microbial fuel cells can be built using CNT.
 - Due to three-dimensional architectures and enlarged electrode surface area for the entry of growth medium, bacteria can grow and proliferate and get immobilized.

• HDTVs

- The resolution of a television, or a monitor, depends greatly on the size of the pixel.
- These pixels are essentially made of materials called "phosphors," which glow when struck by a stream of electrons inside the cathode ray tube (CRT).
- The resolution improves with a reduction in the size of the pixel, or the phosphors.
- Nano crystalline zinc selenide, zinc sulfide, cadmium sulfide, and lead telluride synthesized are used for improving the resolution of monitors.
- The use of Nano phosphors is envisioned to reduce the cost of these displays so as to render High Definition Televisions (HDTVs) and personal computers affordable.
- Next-Generation Computer Chips
 - By achieving a significant reduction in the size of transistors, resistors, and capacitors, the microprocessors which contain these components, can run much faster, thereby enabling computations at far greater speeds

Elimination of Pollutants

- Nano materials are very active in terms of their chemical, physical, and mechanical properties.
- Hence, they can be used as catalysts to react with toxic gases such as carbon monoxide and nitrogen oxide in automobile catalytic converters and power generation equipment to prevent environmental pollution arising from burning of gasoline and coal.

Sun-screen lotions

- Sun-screen lotions containing nano-TiO2 provide enhanced sun protection factor (SPF) as they protect the skin by sitting onto it rather than penetrating into the skin.
- Thus they block UV radiation effectively for prolonged duration and also retain natural skin color while working better than conventional skin-lotions.

• Sensors

Engineered monolayers (few Angstroms thick) on the sensor surface are exposed to the environment and its functionality such as change in potential as the CO level is detected is utilized in sensing.

BIO MATERIALS

- A biomaterial is a nonviable material used in a medical device, intended to interact with biological systems (Williams, 1987)
- A biomaterial is any substance that has been engineered to interact with biological systems for a medical purpose either a therapeutic (treat, augment, repair or replace a tissue function of the body) or a diagnostic one.

Characteristics:

- Physical Hard and flexible materials
- Chemical

It should be non toxic and must not react with any tissue in the body.

CLASSIFICATION OF BIO MATERIALS

• Natural biomaterials

Bio materials which are naturally available.

• Synthetic biomaterial

Bio materials produced in a laboratory or industry by human effort.



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PROPERTIES OF BIO MATERIALS

- Bulk Properties
 - The bulk elastic properties of a material determine how much it will compress under a given amount of external pressure.
 - Relates to the hardness, brittleness, malleability, ductility, elasticity, plasticity and mechanical resistances.
- Surface Properties
 - These are the most important property that a biomaterial possesses.
 - This is due to the fact that, when a device is implanted to tissues, the surface chemistry determine how the material and the surrounding fluid interacts.
 - The surface of metal implant corrode inside the system liberating the metallic ions into the solution.
 - Polymeric materials doesn't corrode, but leach the constituents such as lubricants and monomers from their interiors.

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PROPERTIES OF BIO MATERIALS

- Characterization
 - It is done to obtain detailed information on the physical, chemical, mechanical and surface characteristics.
 - Three methods available to improve the characteristics are;
 - 1. Thermal treatment
 - ✓ The toughness of a biomaterial can be increased by thermal treatment of the material below the melting temperature for a predetermined period of time and this process should be followed by controlled cooling called *annealing*.
 - ✓ The other method is that, the heat treatment step is completed and the alloy is rapidly cooled in a process called *quenching*.
 - 2. Surface improvement
 - ✓ Ammonization is a method in which an oxide film formation is made on the metal.

PROPERTIES OF BIO MATERIALS

- ✓ Nitrating is another method where, oxygen or nitrogen alloys on Aluminum or Titanium base alloys are obtained by placing them in an electrolytic bath and passing electric current.
- ✓ Grinding is a process, that results in surface layer formation which is used to remove surface impurities.
- \checkmark **Polishing** is used to polish the surface of the metal.
- 3. Sterilization
 - ✓ Post manufacturing sterilization is done to remove microorganisms from those materials used for surgical applications.
 - \checkmark This method of dry sterilization will destroy most of the bacteria.
 - ✓ The pathogens are killed by heating at 160° 190°C. This should be followed by moist heat sterilization and is performed in autoclaves.

- □ Heart Valves
- □ Hip replacements
- **D**ental implants
- Artificial tissues Polymer result of condensation of lactic acid and glycolic acid. These are biodegradable.
- □ Vascular grafts must be flexible and are designed as open porous structures
- □ Intra ocular lenses





Two factors to be considered for Biomaterials are;

- Bio functionality refers to the effect of environment on the material and also, the material should satisfy its design purpose.
- Bio compatibility refers to the effect of prosthetic material on the body. It should also consider the cost and rate of production.
- □ Cardio Vascular Application
 - Carbon used in heart valves
 - $\circ~$ Polyurethane for pace makers.



- Ophthalmology
 - Contact lenses
 - Corneal implants
 - Artificial corneas
 - Intraocular lenses

□ Therapeutic Applications

- \circ Orthopaedics
 - Joint replacements (hip, knee)
 - Bone cements
 - Bone defect fillers
 - Fracture fixation plates
 - Artificial ligaments.



Dentistry

- \circ Pins for anchoring tooth implants and also part of orthodontic devices.
- Dentures made from polymeric biomaterials

□ Cosmetic applications

- Artificial skin
- Plastic surgery



Commonly used biomaterials and their applications:

- Silicone rubber Catheters, tubing
- Dacron Vascular grafts
- Cellulose Dialysis membranes
- PMMA Intra ocular lenses, bone cement
- Polyurethanes Catheters, pacemaker leads
- Hydrogels Ophthalmological devices, Drug Delivery
- Stainless steel Orthopedic devices
- > Titanium Orthopedic and dental devices
- Alumina Orthopedic and dental devices
- > Hydroxyapatite Orthopedic and dental devices
- Collagen (reprocessed) Ophthalmologic applications, wound dressings