A Review on Modern Characterization Techniques for Analysis of Nanomaterials and Biomaterials

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Abstract

This review is providing a comprehensive overview of essential genuine characterization techniques for nanomaterial and biomaterials exist in various forms. Nanoscience and nanotechnology is one of the Trans scientific frontier, multidisciplinary and environmentally sustainable research field. Today nanomaterials are widely employing in almost every branches of science and technology. As nanomaterials are invisible and unknown in size, shape, so it is enormously needy the advanced characterizations tools to visualize and analyze the materials at nanoscale. The characterization techniques are of paramount importance in the field of nanoscience and technology. This review is to summarize the present knowledge on the use, advances, advantages and weaknesses of a large number of experimental techniques that are available for the characterization of nanoparticles. Different characterization techniques are classified according to the concept/group of the technique used, the information they can provide, or the materials that they are destined for. Many times the beginner researcher in the domain is not aware with the advanced characterization techniques and its purposes for data interpretation.

Keywords: Nanomaterials, Organic molecules, Bio-materials, Characterization tools, Applications in energy storage devices.

1 Introduction

Nanotechnology combines distinct domain such as classical sciences, mathematics, chemistry, physics, biological sciences, medicine, pharmacology, computer and material sciences which investigate the novel materials, study their properties and find applications in diverse areas [1-3]. The nanomaterials differ in their dimensions, shapes, sizes, compositions, porosity, phases and uniformity and thus several classifications have been used to categorize them [3,4]. To date many types of nanomaterials have been identified which occurs naturally and many new nanoparticles are synthesized artificially. Of course the naturally occurring nanomaterials are more in number than the artificial nanomaterials [5, 6]. The unparalleled potential of engineered submicron structures, which are comparable in the size of biological molecules, has been recognized ever since 1950. But the rapid growth in nanoscience and technology took place after 1950 [1,4, 7]. In 1959, Feynman first introduced the modern concepts of nanotechnology, nanoscience, and nanomaterials and their significance (“There’s Plenty of Room at the Bottom”) [8,9]. Nanoscience takes us deeper because it immerses us
Actually nanomaterials are invisible and it was very difficult to analyze. Thus after the discovery of electron microscope, probe microscope and other characterization techniques revolutionary modifications acquired place in discovery and applications of materials at nanoscale. The advanced characterization technique provides the fast vision and assistances to recognize and classify the materials at nanometer range [10,11]. By the year 2000, nanotechnology was universally acknowledged as a breakthrough innovation and named the sixth rightly revolutionary technology introduced to the modern world. There has been a saying for a long time “Necessity is the mother of invention.” Hence mankind have been passionate enough to nurture science and transform it into productive nanotechnology. Since the dawn of human civilization human beings are using different types of materials to meet their daily needs. Therefore, nanomaterials have always been an integral part of the progress of human civilization [12-13]. Material science is the domain of science and technology where changes are taking place continuously and the particular age of civilization is known after the name of materials in use e.g., stone age about 5000 BC, copper age 5000-800 BC, bronze age 300-00 BC, iron age 800 BC to 40 AD, plastic age since 1907 after the discovery of Bakelite polymer [14]. Thus, it was very clear that material have strong impact on the particular period and gold was probably one of the first elemental metal used by mankind [15]. Furthermore, Gold and silver belong to the family of “metals of antiquity”, having their history with mankind dating back to 6000 BC and 4000 BC, respectively. Gold's brilliance, intrinsic prettiness and capability to preserve its shine for long times made it the most accepted metal in the human history. While there are no clear-cut reports for the discovery of gold, it has always been allied with the gods, with immortality, and with wealth across the human civilizations. Also, some metals have been associated not only with the history of the periodic table, but also irrevocably with the ascent of Homo sapiens. From pre-history to the beginning of the Common Era, only the seven metals, gold, silver, copper, iron, mercury, tin and lead were widely known to man. The evidence of this is obtained from the archeological study of the Indus valley civilization [16-19].

Today material scientists and engineers have focused on tailored materials from the atomic scale upwards to obtain desired properties. A new age in materials known as the tailored material age has been used to describe the revolutionary changes in material science and engineering as well as their impact on society. Since prehistoric times, human have been using fire. The smoke and soot of such fires contained nanoparticles and combustion products. Our early ancestors first learned to make fires, humans have been producing carbon-based nanoparticles. The smoke and soot from their campfires contained nanoparticles known as fullerenes and carbon nanotubes, along with many other combustion by-products. They must have thought the very crude nanoparticle preparations they created were a bit
of a nuisance (depending on how concerned they were about cleanliness), until they decided they could use them in art. [20-21]. Naturally occurring nanomaterials have been intimately associated with life development on the earth from the first cells to human. Nanomaterials and their derivatives have been produced and employed by humans since prehistoric times. However, often ignores the fact that Nature itself is a skilled nanotechnologist, with numerous examples of common nanomaterials literally emanating from natural sources, such as volcanoes and mineral springs but also, in particular, from living organisms. Figure 1 indicates a colorful reminder of such entirely natural sources of nanoscopic and microscopic particles [22]. At the same time, nature also provides the inspiration and eventually also the ingredients— and even some of the methods— for natural nanomaterials. Due to major advancement in characterization techniques, it is becoming possible to better assess all nanomaterials in the earth system and better frame, their long term effects impact on the earth and environment and human health can be estimated [23,24].

Nanomaterials have emerged as an amazing class of materials that consists of a broad spectrum of examples with at least one dimension in the range of 1 to 100 nm. Exceptionally high surface areas can be achieved through the rational design of nanomaterials. Nanomaterials can be produced with outstanding magnetic, electrical, optical, mechanical, and catalytic properties that are substantially different from their bulk counterparts. The nanomaterial properties can be tuned as desired via precisely controlling the size, shape, synthesis conditions, and appropriate functionalization [5, 25,26]. Nanotechnologies involve the manipulation of matter at a very small scale generally between 1 and 100 nano-meters at least in one dimension. Once the materials acquire the nano dimensional size, they develop novel properties and functions. The nanomaterials and nanoparticles may be of different shapes such as nanoparticles, nanotubes, fullerenes, nano-fibers, nano-wires, and nano-sheets etc [27]. A nanomaterial is defined as an “insoluble or bio-persistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nano-meters” as detailed in the recent European Commission Cosmetics Regulation ((EC) No 1223/2009). Thus, nanoscience and technology have been defined by different researchers in different ways. But none of these definitions seems to fit perfectly on the materials at the nanoscale. Therefore, efforts are underway to establish a more comprehensive definition for nanomaterials. Hence, this is a provisional definition until a uniform, European and international definition was made available.
Microscopic and nanoscopic particles are formed by combustion, (a) open fire; (b) Volcanic activity; (c) Precipitation and (d) as bioreductively formed deposits of elements in certain bacteria [22].

In recent years, nanotechnology is an excellent example of an emerging technology, offering engineered nanomaterials with the great potential for producing products with substantially improved performances [28]. Currently, nanomaterials find commercial roles in scratch-free paints, surface coatings, electronics, cosmetics, environmental remediation, sports equipment, sensors, and energy storage devices [29]. The word nano is supposed to be originated from classical Latin nanus or its ancient Greek etonym nanos which means a material whose size has been reduced to $10^{-9}$ m, which is 1000 times smaller than one micron. Thus, it was very clear that nanoscience, nano-engineering, nano-structure, and nanotechnology all these deal with very small-sized objects and systems. Structures on a nano scale are measured at the borderline of the smallest of human-made devices and the largest molecule of a living system [4,8]. Our ability to sustain and manipulate nanostructure will make it possible to exhibit a new physical, biological, and chemical properties of the system that are intermediate in size between single atoms and bulk materials. Moreover, at present the nanotechnology and its associated research discipline together constitute the widespread spectrum of accomplishments towards the promised next industrial revolution [30].

Nanotechnology has been defined in various ways by different scientists and organizations. According to Hunt, “Nanomaterials are an enabling component of the popularly labeled area of nanotechnology”. Nanotechnology as described by Scientific Americana; “The field is a vast grab bag of stuff that has to do with creating tiny things that sometimes just happen to be useful. It borrows
liberally from condensed matter physics, engineering, molecular biology, and large swaths of chemistry” [31-32]. However, a more comprehensive definition was given by the US National Nanotechnology Initiative (NNI) as: (i) research and technology development at the atomic, molecular, or macromolecular levels, approximately 1-100 nanometers (ii) Creation and use of structures, devices, and systems that have novel properties and functions because of their small and intermediate size, and (iii) Ability to control or manipulate on the atomic scale [33]. Another definition created by The Royal Society and The Royal Academy of Engineering is “nanotechnology is the design, characterization, production, and application of structures, devices, and systems by controlling shape and size at the nanometer scale [34]. According to the British Standards Institution Nanotechnology (BSIN) was termed as the design, characterization, production, and application of structures, devices, and systems by controlling shape and size at the nanometer. It was the most promising scientific field of research in decades and also works on the manipulation of materials at macromolecular, molecular, and atomic scales. Thus, the improvement of the properties of materials by controlling their nanoscale structures is the heart of nanotechnology field [35].

The invention of various spectroscopic techniques sped up research and innovations in the field of nanotechnology. IBM researchers developed scanning tunneling microscopy (STM) in 1982, and with STM it became feasible to attain images of single atoms on “flat” (i.e., not a tip) surfaces [36]. Atomic force microscopy (AFM) was invented in 1986, and it has become the most decisive scanning probe microscope technique [36]. The inspiration to manufacture the hard discs with high storage density stimulated the measurement of electrostatic and magnetic forces. This led to the development of Kelvin-probe-, electrostatic-, and magnetic-force microscopy [37]. Currently, nanotechnology is rapidly evolving and becoming part of almost every field related to materials chemistry. The field of nanotechnology is evolving every day, and now powerful characterization and synthesis tools are available for producing nanomaterials with better-controlled dimensions and other nanomaterials related properties too.

1.1. Developmental stages of Science at Nanoscale:

However, the existence and use of nanoparticles are goes back in history long before that. Many types of material exit naturally at the nanoscale. Scientists think that nanoparticles and nanostructured materials are originated during Big Bang process from materials leasing to the formation of the universe and earth. The first relationship between human life and nano-scale was developed in Ayurveda, which is about 5000 years old the Indian system of medicine. Ayurveda had some knowledge of nanomedicine synthesis and its effects even before the term nanotechnology was coined [38]. The process of formation of bhasmas, a kind of metallic nano-particle is well described in ancient Ayurveda literature Ras
Ratnakar authored by Nagarjun in 50 BC, clearly describes the process of formation of Bhasma which is a special kind of Ayurvedic medicine. A combination of traditional method and new scientific method formulate a new approach to metal nanoparticles. The physical and chemical method can produce pure and well-defined nanoparticles, it is quite expensive and dangerous to the environment and not eco-friendly [39]. Ayurveda describes this process as shorthand i.e. purification and Maran i.e. killing the metallic properties so that it becomes digestible and suitable for medicinal use. Also, the modern therapy of medicine Homeopathy developed by Dr. Samuel Hahnemann uses an alcoholic solution of metallic nanoparticles for curing diseases. In the Homeopathic book “Organon of Medicine”, Dr. Samuel Hahnemann mentions several homeopathic medicines in which metal is in nano form e.g. Aurum metallicum (nano Gold), Argentium (nano Silver), etc. [40]. Homeopathic way of treatment has a strong belief that by lowering the concentration the property changes and the product can be used to cure diseases.

History claims that nanoparticles have been around us for a long time. Presumably, the use of nanoparticles was reported in 1570 as aurum potable (potable gold) and Luna potable (potable silver) which alchemists used as elixirs. Unfortunately, they did not make the consumer immortal, as is evident by the fact that those alchemists are not among us today. However, one of the oldest applications of nanoparticles that we come across in the literature is the use of gold nanoparticles for staining glasses, a famous example of which is the Lycurgus cup which dates back to the 4th century AD [41]. The origin of unusual optical properties of nano-structured materials however could be dated back to the 17th century when the brilliant color of some of these nanoparticles was used advantageously to make stained glass windows of cathedrals. Faraday explained the origin of their color owing to the presence of metallic gold nanoparticles, especially in colloidal form. Moreover, Gustav Mie (1868-1957) approached this problem by working out the solution to scattering of light by small spheres, starting with Maxwell’s equations. His approach is all the more remarkable because, at the time, the importance of Maxwell’s equations was not yet recognized by all physicists. His classic paper, Mie (1908), is titled “Beiträge zur Optik trüber Medien, speziell kolloidaler Metalllösungen,” or “Contributions to the optics of turbid media, particularly colloidal metal solutions.” Mie used his solution equations to explain how particle size and absorption properties can explain the different colors [42]. Figure 2 indicates an example of red to violet colors in suspensions of gold particles. The difference in colors is due to the different sizes of the gold particles, which are smaller than the wavelength of visible light. Understanding the optical effects of small concentrations of very small particles had important industrial uses due to addition of metallic nanoparticles to molten glass was a common path to develop glass of various colors [43].
Fig. 2. Suspensions of gold nanoparticles of various sizes showing a range of colors. Photo by Shivanand Teli from en.wikipedia.org/wiki/Colloidal.gold [42].

However, research and invention on nanomaterials has received more attention only during the last few decades. This is because of the growth of the electron microscope which could expose the structure of nanomaterials. In the existing age indeed, the nanotechnology mania is across-the-board through essentially all the arenas of science and technology and the public is almost experiencing the meaning of the quote by Nobel Laureate Richard Smalley: “Just wait—the next century is going to be incredible. Soon, we are about to build things that work on the smallest possible length scales, atom by atom. These little nano-things will revolutionize our industries and our lives” [43]. This is becoming more and more marked in the form of potential applications of nanoparticles which encompass to a wide range of areas such as catalysis, biosensors, solar cells, supercapacitors, smart windows, and medical sciences. A remarkable aspect of nanomaterials is that several factors can influence their properties such as size, shape, surface composition and larger surface-to-volume ratios, dielectric environment, reduced imperfections and inter-particle interactions [44, 45]. The chronological development of science at the nanoscale is depicted in Table 1.

<table>
<thead>
<tr>
<th>Period</th>
<th>Development in Nanoscience and Technology</th>
</tr>
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<tbody>
<tr>
<td>5000 years ago</td>
<td>Ayurveda system of medicine describes the use of nanoparticles in medicine</td>
</tr>
<tr>
<td>2000 years ago</td>
<td>Sulfide nanoparticles were used by Greek and Romans to dye the hair</td>
</tr>
<tr>
<td>4th Century</td>
<td>Lycurgus cup is a dichromatic glass</td>
</tr>
<tr>
<td>6th Century to 15th century</td>
<td>Stained window glass</td>
</tr>
<tr>
<td>Century Range</td>
<td>Event/Discovery</td>
</tr>
<tr>
<td>--------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>9th Century to 17th century</td>
<td>Ceramic glaze</td>
</tr>
<tr>
<td>1000 years ago</td>
<td>Gold nanoparticles of different sizes and shapes were used to color the glasses.</td>
</tr>
<tr>
<td>13th Century to 18th century</td>
<td>Damascus sword</td>
</tr>
<tr>
<td>1857</td>
<td>Michel Faraday synthesized a colloidal solution of gold</td>
</tr>
<tr>
<td>1905</td>
<td>Einstein published his study on the dimension of a sugar molecule is ≈ 1 nm</td>
</tr>
<tr>
<td>1936</td>
<td>Erwin Muller developed Field Emission Microscope</td>
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<tr>
<td>1947</td>
<td>Semiconductor was discovered by John Bardeen, William Shockley and Walter Brattain</td>
</tr>
<tr>
<td>1950</td>
<td>Theory and process of growing monodispersed colloidal material was developed by Victor La Mer and Robert Dinegar</td>
</tr>
<tr>
<td>1951</td>
<td>Erwin Muller discovered Field Ion Microscope</td>
</tr>
<tr>
<td>1956</td>
<td>Molecular engineering term was coined by Arthur von Hippel</td>
</tr>
<tr>
<td>1958</td>
<td>First Integrated circuit was developed by Jack Kilby</td>
</tr>
<tr>
<td>1959</td>
<td>R. Feynman, in his lecture at the annual meeting of the American Association of Physical Sciences, claims that <em>There is Plenty of Room at the Bottom</em></td>
</tr>
<tr>
<td>1965</td>
<td>Moore’s law was developed</td>
</tr>
<tr>
<td>1974</td>
<td>Norio Taniguchi introduced the term “Nanotechnology”</td>
</tr>
<tr>
<td>1981</td>
<td>E. Drexler designs molecular machines that mimic enzymes and ribosomes</td>
</tr>
<tr>
<td>1981</td>
<td>Scanning Tunneling Microscope was developed by Gerd Binning and Heinrich Rohrer</td>
</tr>
<tr>
<td>1981</td>
<td>Nano-crystalline semiconducting quantum dots were discovered</td>
</tr>
<tr>
<td>1984</td>
<td>1984 The first description of the term “Dendrimer” by D.A. Tomalia and the preparation method of PAMAM dendrimers</td>
</tr>
<tr>
<td>1985</td>
<td>Buckminster Fullerene was invented</td>
</tr>
<tr>
<td>1985</td>
<td>Colloidal semiconductor nanocrystals quantum dots are developed</td>
</tr>
<tr>
<td>1986</td>
<td>Atomic Force Microscope (AFM) was developed</td>
</tr>
<tr>
<td>1986</td>
<td>Engines of Creation First book on Nanotechnology by K Eric Drexler</td>
</tr>
<tr>
<td>1989</td>
<td>Manipulation of individual Xenon atoms to spell IBM logo</td>
</tr>
<tr>
<td>1990</td>
<td>Early nanotechnology based companies commences to produce products</td>
</tr>
<tr>
<td>1991</td>
<td>The discovery of Carbon nanotubes (CNTs)</td>
</tr>
<tr>
<td>Year</td>
<td>Event</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
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<tr>
<td>1992</td>
<td>Nanostructured catalytic materials MCM-41 and MCM-48 were discovered, which are now using in refining of various kinds of crude oil</td>
</tr>
<tr>
<td>1994</td>
<td>Invention begins on Drug delivery systems</td>
</tr>
<tr>
<td>1995</td>
<td>FDA approved Doxil (Liposomal Doxorubicin)</td>
</tr>
<tr>
<td>1997</td>
<td>FDA approved AmBisome (Liposomal Amphotericin B)</td>
</tr>
<tr>
<td>1998</td>
<td>First time applied DNA nanoparticles for controlled gene delivery</td>
</tr>
<tr>
<td>2000</td>
<td>The first FDA approval of medicinal product based on the technology of Liquid Crystals (NanoCrystal Technology) and the solid dose formulation of the immunosuppressant sirolimus- Rapamune</td>
</tr>
<tr>
<td>2005</td>
<td>FDA approves Abraxane®, the nanotechnological formulation of paclitaxel</td>
</tr>
<tr>
<td>2008</td>
<td>In the market: PEG-Certolizumab pegol (trade name, Cimzia) anti-TNF Fab for rheumatoid arthritis and Crohn’s disease</td>
</tr>
<tr>
<td>2012</td>
<td>Biomimetic drug delivery systems: the first publication in the literature, July 2015 successful clinical trials of ThermoDox (lyso- thermosensitive liposomal doxorubicin )</td>
</tr>
<tr>
<td>October 2015</td>
<td>FDA approves Onivyde (irinotecan liposomal) for advanced pancreatic cancer</td>
</tr>
</tbody>
</table>

### 1.2. Classification of Nanomaterials

Nanomaterials, naturally present in the earth, move through different compartments i.e. biosphere, lithosphere, atmosphere and hydrosphere within the natural biogeochemical cycle. There are various ways to classify the materials at nanoscale nature is the best manufacture of nanomaterials and nanostructure. Natural nanomaterials mean the materials that belong to the natural world without human modification or processing. The natural nanoparticles are generated in different environmental compartment by different types of physical, chemical and biological processes. The nano-sized particles, regardless of their origin, participate in same biochemical processes \([5,6, 8, 46]\). The fate of behavior of engineered nanoparticles are similar to their natural counterparts with same compositions. Irrespective of their origin the nanoparticles possess higher number of atoms on the surfaces. The engineered nanoparticles are less commonly poly-dispersed. The surface of anthropogenic nanoparticles are modified in the environment and living systems, through the adsorption and desorption of organic and inorganic compounds of natural or anthropogenic origin. Due to their structure, they have remarkable properties because of their inherent nanostructure, herein we will try to focus on some types of classifications of nanomaterials \([47-48]\).
1.2.1 Classification based upon the origin of nanoparticles:

Natural nanomaterials can be found in a variety of forms in nature, including viruses, protein molecules, minerals like clay, natural colloids like milk and blood (liquid colloids), fog (aerosol type), gelatin (gel type), mineralized natural materials like shells, corals, and bones, insect wings and opals, spider silk, lotus leaves, gecko feet, volcanic ash, and ocean spray [49]. On the other hand, Artificial nanomaterials such as carbon nanotubes and semiconductor nanoparticles like quantum dots (QDs) are examples of artificial nanomaterials that are made consciously using precise mechanical and manufacturing procedures. Nanomaterials are categorized as metal-based materials, dendrimers, or composites depending on their structural makeup [48, 50-51]. The classification of nanomaterials based upon origin and artificial are presented in Table 2.

Table 2. Classification of Nanoparticles based on Origin

<table>
<thead>
<tr>
<th>Anthropogenic or Engineered Nanoparticles</th>
<th>Incidental Particles</th>
<th>Natural Particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanotubes, Fullerenes</td>
<td>Combustion products</td>
<td>Plants, trees, clays, bones etc</td>
</tr>
<tr>
<td>Metal oxides</td>
<td>Industrial process</td>
<td>Erosion</td>
</tr>
<tr>
<td>Quantum dots</td>
<td>Vehicle emission</td>
<td>Dust, Forest fire product, volcanic ashes, desert surfaces, dust from comic bodies, ocean spray, radioactive decay and weathering processes of metal or anion containing rocks.</td>
</tr>
<tr>
<td>Nanotubes</td>
<td>Construction</td>
<td>Viral Nanoparticles</td>
</tr>
<tr>
<td>Nanowires</td>
<td></td>
<td>Cowpea mosaic virus, cowpea chlorotic mottle virus, canine parvovirus, and bacteriophage</td>
</tr>
<tr>
<td>Dendrimers</td>
<td></td>
<td>Oxides and oxyhydroxides of iron, manganese and aluminium and aluminosilicates, humic substances, various bio-polymers synthesized and released.</td>
</tr>
</tbody>
</table>

1.2.2 The dimensionality of Nanomaterials

The nanoparticles can be classified based on their dimensionality. Nanomaterials are materials with one external dimension that measures 100 nm or less or with internal structures measuring 100 nm.
or less. The nanomaterials have the same composition as known materials in the bulk form but differ in their physicochemical properties. Based on their dimensionalities, nanomaterials are placed into four different classes with examples are represented in Fig.3 [51,52].

a) **Zero Dimensional Nanomaterials (0-D):** In this type of nanomaterial all the three dimensions i.e. x, y, and z lie within the nano range i.e. 1 to 100 nm and are not dimensional outside the nanometer metric. Examples such as molecules, quantum dots, fullerenes, clusters, fullerenes, rings, metal carbides, powders, and grains, etc. The zero dimensional nanomaterials could be crystalline or amorphous in nature. Such type of materials exhibits various shapes and size and they can be metallic or ceramic nanomaterial.

b) **One Dimensional Nanomaterials (1-D):** In this type of nanomaterial out of three dimensions two dimensions i.e. x and y lie within the nano range i.e. 1 to 100 nm and one dimension z is outside the nano scale. The Examples like nanotubes, nano-rods, thin films, needle shaped nanomaterials, nanowires, fibers, filaments, whiskers, spirals, belts, springs, columns, and needles, etc.

c) **Two Dimensional Nanomaterials (2-D):** In this type of nanomaterial out of three dimensions one dimension i.e. x lies within the nano range i.e. 1 to 100 nm and two-dimensional i.e. y and z are outside the nano range. Examples including layers, coatings, nano-walls, nano-sheets, free particle tubes, ultrafine grained over layer, wires and plates, nano wells, nanofilms, and nanocoatings etc.

d) **Three Dimensional Nanomaterials (3-D):** In this type of nanomaterial all the three dimensions i.e. x, y and z lie outside the nano range i.e. 1 to 100 nm. In a true sense, these are not nanomaterial but are bulk materials. Examples are bulk powders, bulk nanomaterials, bundles of nanowires and nanotubes and multi-nano layers, dispersion of nanoparticles, bundles of nanowires and nanotubes as well as multinanolayers.
1. 2.3 Classification Based on Molecular structure

(a) Organic Nanomaterials: In recent years, engineered nanoparticles have raised extensive interest because of their promising medical usage in vaccination, diagnostic imaging procedures, or sustained delivery of drugs. Meanwhile, nature provides a wide range of examples of organic nanoparticles and template on natural or synthetic molecules. Organic nanoparticles differ from inorganic nanoparticles in terms of principle of fabrication. There is international resurgence of interest in natural products for the development of novel drugs and therapies that could be possible applications in various domains [53,54]. The inorganic nanomaterials are generally synthesized through precipitation techniques and effect of bonding contrast such as covalent and metallic etc. The adjoining organic nanoparticle to inorganic nanoparticle was the dendrimer, which was single molecule nanoparticle. The organic nanoparticle including dendrimers, proteins, lipids, virus, chitosans, liposomes, micelles, etc. Most of the organic nanoparticles are obtained by many organic molecules which are determined together by self-organization or chemical bonding. Self-assembly and the presence of zwitterion molecules, with polar and non-polar regions, as the main component of nanoparticles or means to encapsulate other organic molecules are key elements for the fabrication of many of the organic nanoparticles [55].

Last few decades, research are concentrated on development of protein nanoparticles as drug delivery vehicles. Casein recognized as a safe protein is a major milk protein that forms an integral part of the daily diet. It contains many remarkable properties that make it a good candidate for conventional
and novel drug delivery systems. Casein nanoparticles may be alternative to albumin as a matrix for drug delivery because it is inexpensive and has better amphiphilicity, good dispersibility, and rapid reconstruction in an aqueous solution [56]. It is interesting to note about carbon, whether it is organic nanomaterial or inorganic nanomaterial. The correct answer is, carbon nanomaterials, liposomes, polymeric micelles and dendrimers all are examples of organic nanomaterials. But it should be noted that few types of carbon based nanomaterials like diamond and graphite nanomaterials are inorganic in nature. Today there is ever increasing interest in the development of organic nanomaterials for biomedical applications in veterinary medicine, health sciences and agriculture. So far several types of chitosan nanoparticles are used in medicine because of its low solubility in neutral and alkaline pH. Chitosan based many reports focused on their controlled delivery as well as their ability to support regeneration of oral tissues with uses that extent almost all fields of dentistry: endodontics, periodontics, regenerative dentistry, invasive dentistry or even implantology, veterinary medicine and health sciences are mentioned in Table 3 [57-60].

**Table 3.** Applications of Chitosan based Nanomaterials

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Chitosan nano-system</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bone morphogenetic protein-2</td>
<td>Bone regeneration (2015)</td>
</tr>
<tr>
<td>2</td>
<td>Bone morphogenetic protein-7</td>
<td>Bone regeneration (2015)</td>
</tr>
<tr>
<td>3</td>
<td>Protein growth factor</td>
<td>Bone regeneration (2014)</td>
</tr>
<tr>
<td>4</td>
<td>Dexamethasone</td>
<td>Bone regeneration (2015)</td>
</tr>
<tr>
<td>5</td>
<td>Cetylpyridinium chloride and naf</td>
<td>Dental toothpastes (2015)</td>
</tr>
<tr>
<td>6</td>
<td>Chlorhexidinedihydrochloride</td>
<td>Dental toothpastes (2015)</td>
</tr>
</tbody>
</table>

**b) Inorganic Nanomaterials:** Inorganic nanomaterials are purely made of metal precursors, they can be monometallic, bimetallic [61] or polymetallic [62]. Bimetallic NPs can be made from alloys or formed in different layers (core–shell) [61]. Due to the localized surface plasmon resonance characteristics, these nanomaterials possess unique optical and electrical properties [4]. In addition, some metal nanomaterials also possess unique thermal, magnetic, and biological properties [63]. This makes them increasingly important materials for the development of nanodevices that can be used in numerous physical, chemical, biological, biomedical, and pharmaceutical applications [64, 65] (these applications are discussed in detail later in the applications section of the review). In present days, the size-, shape-, and facet-controlled synthesis of metal nanomaterials is important for creating cutting-edge materials [66].

Semiconductor nanomaterials are made of semiconductor materials, which possess properties between metals and non-metals. According to different sources, inorganic nanomaterials can be grouped
into two main classes (1) Non-metallic nanomaterials. Examples like silica-based nanomaterials, and (2) Metallic nanomaterials e.g. silver, gold nanomaterials, etc. The inorganic nanomaterials can be made by crystallization of inorganic salts forming the three dimensional arrangements. Because of their diverse chemical composition, inorganic nanoparticles have wide range of bio-distribution, accumulation and toxicity. The colloidal inorganic nanoparticles are exhibiting various applications in
various biomedical area such as veterinary medicine, agriculture and health etc [67,68].

1.2.4 Nanocomposites: These are a class of nanomaterials wherein one or more phases at nano-sized dimensions (zero, one, and two-dimensions) are embedded in ceramic, metal, or polymer materials. These can be made by inorganic or organic components at the molecular level to obtain new properties. These are high performance material exhibit unusual property combinations and unique design possibility. The nanocomposites are looked as an alternative to the overcome the limitations of micro-composites and mono-lithic, while posing preparation challenges related to control of elemental composition and stoichiometry in the nanocluster phase [69]. The nanocomposites can be further subdivided into three main types such as (1) Metal matrix nanocomposites: Like the other types of composite materials, metal matrix composite materials contain that least two phases which are physically and chemically distinct. None of the phase can separately exhibit all the properties of composite material. The composites made from Al matrix with silicon carbide particles are used in automobile industries, thermal management, aerospace applications etc. Thus, these nanocomposites are highly suited for the creation of materials with high service temperature and compression strength. Therefore, such materials can be significantly and suitably used for high temperature and shear/compression strength [70.71]. (2) Polymer matrix nanocomposites: This is the most common type of nanocomposite material. Here isolated nanoscale particles are distributed in the bulk or body of polymers. The polymer matrix is used to disperse agglomerated nanoparticles in reality. The functional nanoparticles with superior physical and chemical properties gives the new possibility in micro optics, energy conservation, electronics and energy storages devices such as batteries and super-capacitors, and functional textiles [72,73]. (3) Ceramic matrix nanocomposites: The ceramic matrix nanocomposites are aluminum oxide, silicon carbide systems which have potential applications in diverse fields. The experimentations have shown that addition of up to 10% of silicon carbide in the aluminum oxide of a particular size enhances the properties of materials up to the considerable extent. The nanocomposites can be manufactured through a variety of ways. The conventional method is the powder method, which includes the vapor techniques such as chemical vapor deposition, physical vapor deposition, spray pyrolysis, polymer precursor routes. The chemical route of nanocomposite synthesis are colloidal and precipitation techniques, sol-gel process, template synthesis etc. The sol-gel process can be affected by
a wide range of factors which permit control of the structure and chemical properties of the final material [74]. The nanocomposites are broadly used in medical and drug and found that the therapeutic efficacy of nanocomposites are enhanced. Owing to their distinctive optical properties the nanocomposites are used in biological, veterinary and medical imaging techniques. These nanocomposites can produce efficient contrast. On the other hand, the packaging and food processing industries have indicated nanotechnology as the next great thing. Nanocomposites are used in medical industries in various ways such as adhesives, coatings and lubricants as verified by their mechanical properties which includes stress, strain and young modulus properties. The superior properties lead to better possibility of formation of nano-devices. The tribological of polymers can be effectively improved by incorporating the materials at nanoscale [75, 76].

1.2.5 Carbon-Based Nanomaterials: The carbon-based nanomaterials include fullerene, single-walled carbon nanotubes (SWCNTs), and multiple-walled carbon nanotubes (MWCNTs). These are a novel class of nanomaterials that are broadly used in biomedical fields like the delivery of therapeutics, and biomedical. Owing to exceptional structural, mechanical, electronic, and optical properties carbon nanotubes are considered as new-generation nano-probes [77, 78]. The carbon based nanomaterials display interesting unique physical, chemical and structural properties are useful for biological uses particularly in veterinary sciences and animal husbandry. Concerning their toxic effects in the biological system, several chemical modification strategies have been developed and successfully used in biomedical applications such as drug delivery, cancer therapy, detection of biomolecules, tissue engineering etc [79, 80].

1.2.6 Dendrimers: Dendrimers are nano-sized polymers built from branched units, radially symmetric molecules with well-defined, homogeneous, and monodisperse structure that has a usually symmetric core, with inner and an outer shell. There are three traditional macromolecular architectural classes are broadly renowned to produce rather polydisperse products of different molecular weights. A variety of dendrimers exist, and each has biological properties such as polyvalency, self-assembling, electrostatic interactions, chemical stability, low cytotoxicity, and solubility [81]. The surface of dendrimers has numerous chain ends, which can be tailored for a particular biological and chemical functions. Due to such characteristics the dendrimers can be used as a potent catalyst to change the rate of chemical reactions. Also, the three dimensional dendrimers are having interior cavities into which some another molecules of suitable size can be fit. Therefore, the specific types of dendrimers can be used as potent drug delivery vehicle [82].
1.2.7 Ceramic Nanomaterials: Ceramic material is one of the most common type of material used by mankind in modern period, today we use various type of ceramic materials in our day today life which includes glassware, china clay materials, earthen pot materials used to store water, cement in construction line and many more. The nano-ceramics is a new type of ceramic material which is developed since 1970s. The ceramic nanomaterials can maintain their stability and properties at higher temperature. These materials are having high surface to volume ratio. These nanomaterials can be successfully applied in various ways such as photo catalyst, photo degradation of dyes, drug delivery, and imaging. Certain types of ceramic nanomaterials exhibit unique optical and magnetic properties such as luminescence and magnetoresistance which is useful in many applications such as sensors and data storage devices [83]. The ceramic based nanomaterials especially calcium phosphates and BGN () have been proven to be effective in bone tissue regeneration and have potential applications in different medicine [84]. Also they can be used as a potent catalyst in chemical transformation reactions and dielectric material. In environment science, ceramic nanoparticles can be used in adsorbent for removing pollutants from air and water. Nanoceramic materials have gradually become engineering materials that can replace traditional industries such as catalysis, coatings and electronic devices [85].

1.2.8 Metal Nanoparticles: This is probably the most extensively worked branch in nanoscience and technology. This type of nanoparticle is generally made up of single type of metal. Today we generally synthesize metallic nanoparticles from bottom up route of synthesis. In the bottom up synthesis of metallic nanomaterials we are using metal salts as the precursor. The metal salt is dissolved in suitable solvent generally in aqueous medium. This results in the formation of cation and anion. The cation is reduced using suitable electron donor. The supported metal nanoparticle based catalytic solids typically consists of high surface to volume ratio [86]. Metallic nanoparticles have different applications in different types of field. Noble metal nanoparticles have been used for several biomedical applications such as anticancer, radiotherapy enhancement, photo-ablation therapy, hyperthermia, drug delivery, thermal ablation, antibacterial assay, antifungal, gene delivery etc. The metal based nanoparticles such as silver, copper, gold, iron, zinc and platinum attracted attention of researchers for their uses in veterinary medicine and health science. Apart from these applications, these nanoparticles find applications in research areas, detection and imaging of biomolecules, and in the environment and bioanalytical applications [87].

1.2.9 Semiconductor Nanoparticles: Semiconductor nanoparticles have attracted much attention because of their unique properties such as quantum size effects, non-linear optical properties and luminescence etc. Semiconductor have properties like those of metals and also like non-metals. Thus
they have intermediate properties. When the size of semiconducting material is reduced up to nanoscale, they are known as “Semiconducting Nanomaterials” [88]. These particles have a wide bandgap, which on tuning shows different types of properties. They are wide as a photo-catalyst, in electronic industries, photo-optics, and water splitting applications. There is considerable interest in semi-conductor nanoparticles trapped in sol-gel silica from the view point. The semiconducting nanoparticles have interesting physical and chemical properties when compared with their conventional bulk counterparts. Narrow and intensive spectra, continuous absorption band, high chemical and photo-bleaching activity stability, process ability and surface functionality are among the most attractive properties of the materials [89].

1.2.10 Lipid based nanoparticles: Over the past decades, the therapeutic potential of nanomaterials as novel drug delivery systems complementing conventional pharmacology has been acknowledged. Among these nanomaterials, lipid-based nanoparticles (LNPs) have shown remarkable pharmacological performance and promising therapeutic outcomes, thus gaining substantial interest in preclinical and clinical research. This type of nanoparticle is generally spherical with diameter ranging between 10 to 100nm. It consists of the solid core which is made up of lipids and the matrix containing soluble lipophilic molecules. Lipid based nanoparticles have applications in the biomedical field as the drug carrier and RNA release therapy in cancer treatment [90].

1.2.11 Polymeric Nanoparticles: Polymeric nanoparticles are organic-based nanoparticles. Depending upon the method of preparations, these have structures like nano-capsules or nano-spheres. A nano-sphere particle has a matrix-like structure whereas nano-capsules particles have core-shell morphology. The polymeric nanomaterials can protect drugs and control their release of the drug. They have a wide range of applications in drug delivery and diagnostics [91]. Cancer is second leading cause in the world. In coming few years, it is expected that cancer cases will increase globally. At present for the management of cancer the recommended therapies are surgery, chemotherapy and radiation. However, inspite of the existing methods of treatment there is high mortality and morbidity due to cancer. The resultant systematic toxicity and adverse effects highly limits the tolerated doses of anti-cancer drugs and thus restricts its therapeutic efficacies [92]. The toxicity of conventional chemotherapeutic drugs and its indiscriminate destruction of healthy and rise of multidrug resistance is a big problem in front of veterinarians and health professionals. Thus, researchers over the planet are working day and night to find the solution this severe problem. The main difficulty is to improve the selectivity of anti-cancer drug for tumor cells and its surroundings. Thus, the targeting the tumor tissue by nano-medicine may
be an effective route of treatment. The nano-particulate formulation can overcome this limitation by providing alternate pathway of cellular internalization [93].

Few decades earlier the nanomaterials become extremely attractive for the application in biology and medicine. The nanomaterials have the potential to modulate biopharmaceutical features, therapeutic efficacy of encapsulated drugs. The polymer based nanoparticles are colloidal systems made up of natural and synthetic polymers. They are significantly advantageous over other types of nano-carriers such as micelles, liposomes, inorganic nanostructures etc. Here it is worth to mention that several chemotherapeutic drugs have been encapsulated in the polymers so as to enhance the therapeutic efficacy of the drug. In the earlier attempt to use polymers as drug carriers, the non-biodegradable polymers such as poly methylacrylates were used [94]. Generally, the non-biodegradable polymers require longer time than their effective duration of applications. The degradation rate of biodegradable polymers is affected by several factors such as temperature, pH of the medium etc. The biodegradable polymers are generally synthetic polymers. The biopolymers are very important part of classical biomaterials and is widely used in biomedical applications [95]. The natural biopolymers used are of animal origin used for the development of pharmaceutical formulations. The commonly used natural biopolymers are albumin, gelatin, chitosan, hyaluronic acid. Chitosan is the most widely used cationic polysaccharides approved by food and drug administration. The chitosan polymers are degraded by different enzymes such as lysozymes, chitosan’s, cellulose, lipase and pectinase. Alginate is the anionic polymer derived from marine algae. Carrageenan is another sulfated polysaccharide, which carriers a high negative charge and is derived from seaweed. The drug encapsulated in PNs are released by means of diffusion through the polymeric network, erosion of the matrix material, hydrostatic swelling or by combination of all these factors [96].

2. Characterization techniques

Without tool to characterize nano materials, it would be blind to the nanoscale. Methods to measure atomic scale phenomenon essentially predates the formal designation of nanoscience as a new path in scientific exploration. Of course, a technique that measures macroscopic objects around since the beginning of intellectual thoughts. One of major distinction to keep in mind is that to measure macroscopic properties needed to microscopic properties at nano level. Characterization methods of the past have extracted molecular and atomic information by averaging the bulk response to stimulation. This kind of methodology also applies to the analysis of nanomaterials. The need to sort, name, categorize, catalog and detail the things, parts and components of our world has accompanied humanity since the dawn of civilization. Characterization and reporting are fundamental to science [97].
Measurements are accompanied by tools: the instruments, machines, equipment and computer hardware and software. There is much type of characterization methods and most predate the advent nanoscience and nanotechnology. Some methods have actually evolved alongside nanoscience and helped launch the nano age itself. Measurement is accompanied by fundamental to science. They are the means by which we communicate our scientific achievements. Measurement is characterization, the language because of extremely minute size nanoparticles are invisible to human eyes. The magnification power of human eye is up to $10^3\text{M}$ and its resolving power is $1'$. Thus nanoparticles cannot be directly visualized and the size and shape of nanoparticles determines its activity [98].

Electrons are able to interact with matter in different ways. In all cases, electrons serve as the primary probe. An electron impinging on a solid surface may be scattered once, several times, or not at all. Multiple scattering occurs if the dimension of the material is greater than approximately twice the mean free path of electron. The scattering may be elastic or inelastic and forward or backward [99]. The probability of scattering is determined by the scattering cross-section and mean free path of electrons. When a high energy beam of electron is incident upon a thick specimen, a pear shaped region known as interaction volume is formed. The interaction volume which increases with increasing beam voltage and decreases with increasing specimen density is normally between 1 and 5μ. In general electrons are able to penetrate solid materials to a depth proportional to $E^2$ [100]. Thus it can be indirectly analyzed by advanced instrumental methods. The instruments used for analysis is of two types i.e. 1) Spectroscopes and 2) Microscopes, respectively.

2.2 Spectroscopy: It is branch of science which deals with the interaction of electromagnetic radiations with matter. Also most powerful tool available for the study of atomic and molecular structure and is used in analysis of a wide range of samples. Electromagnetic radiations are widely used for spectroscopic techniques for characterization of various nanomaterials and biomaterials [101,102] and details are summarized in Table 4.

<table>
<thead>
<tr>
<th>Electromagnetic Radiations</th>
<th>Applications</th>
<th>Orientations</th>
<th>Frequency</th>
<th>Wave Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma rays</td>
<td>Ionization and Gamma garden</td>
<td>Nuclear</td>
<td>$10^{21}$</td>
<td>$3\times10^{-11}$</td>
</tr>
<tr>
<td>X rays</td>
<td>XRD</td>
<td>Removal of inner shell electrons</td>
<td>$10^{19}$</td>
<td>$3\times10^{-9}$</td>
</tr>
<tr>
<td>Ultraviolet rays</td>
<td>UV-visible spectroscopy</td>
<td>Removal of outer shell electrons</td>
<td>$10^{17}$</td>
<td>$3\times10^{-7}$</td>
</tr>
</tbody>
</table>
Characterization refers to the study of a material’s features such as its composition, structure, and various properties like physical, electrical and magnetic, etc. The particle can be spherical, tubular, or irregularly shaped and can exist in fused, aggregated, or agglomerated forms. Two of the main parameters studied in the characterization of nanoparticles are size and shape along with size distribution, degree of aggregation, surface charge, and surface area and to some extent evaluate the surface chemistry. Size, size distribution, and organic ligands present on the surface of the particles may affect other properties and possible applications of nanoparticles. The various instrumental methods work on different principles. Herein an effort has been made to classify the instrumental methods based on the physical properties to be measured as enlisted in Table 5 [103].

**Table 5. Instrumental Methods based upon Physical Property Analysis**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Physical Properties</th>
<th>Instrumental Methods Based upon Physical Property</th>
</tr>
</thead>
</table>
| 1      | Absorption of Radiation       | • Spectrophotometry X-Ray, UV, Visible, IR, colorimetric  
• Atomic Absorption  
• Nuclear Magnetic Resonance  
• Electron Spin Resonance Spectroscopy (ESR) |
| 2      | Emission of Radiation         | • Emission Spectroscopy X-Ray, UV, Visible  
• Flame photometry |
| 3      | Rotation of Radiation         | • Polarimetry  
• Optical Rotatory  
• Dispersion  
• Circular Dichroism |
| 4      | Diffraction of Radiation      | • X-Ray |
Herein we are trying to enlist the characterization and information, which was get after analysis in tabular form. After this the commonly used characterization techniques are discussed in detail as presented in Table 6, which provides the information regarding various characterization techniques and the main information obtained from them [104].

**Table 6. Modern Characterization Techniques for Analysis of Nanomaterials and Biological Materials**

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Abbreviation</th>
<th>Characterization Techniques</th>
<th>Main Information (Utility)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>XRD</td>
<td>X-Ray Diffraction</td>
<td>Crystal structure, composition, crystallite size,</td>
</tr>
<tr>
<td>2</td>
<td>XAS</td>
<td>X-Ray Absorption Spectroscopy</td>
<td>X-ray absorption co-efficient, chemical state of species, interatomic distances, Debye-Waller factors, and non-crystalline NPs</td>
</tr>
<tr>
<td>3</td>
<td>SAXS</td>
<td>Small Angle X-Ray Scattering</td>
<td>Particle size, size distribution, growth kinetics</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>4</td>
<td>XPS</td>
<td>X-ray photoelectron Spectroscopy</td>
<td>Electronic structure, elemental composition, oxidation states, ligand binding</td>
</tr>
<tr>
<td>5</td>
<td>FT-IR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
<td>Surface composition, ligand binding</td>
</tr>
<tr>
<td>6</td>
<td>NMR</td>
<td>Nuclear Magnetic Resonance Spectroscopy</td>
<td>Ligand density and arrangements, electronic core structure, atomic composition, the influence of ligands on NP shape, NP size</td>
</tr>
<tr>
<td>7</td>
<td>BET</td>
<td>Brunauer Emmett Teller</td>
<td>Surface area</td>
</tr>
<tr>
<td>8</td>
<td>TGA</td>
<td>Thermogravimetric Analysis</td>
<td>Mass and composition of stabilizers</td>
</tr>
<tr>
<td>9</td>
<td>LEIS</td>
<td>Low Energy Ion Scattering</td>
<td>Thickness and chemical composition of self-assembled monolayers of NPs</td>
</tr>
<tr>
<td>10</td>
<td>UV-Visible spectroscopy</td>
<td>Ultra-violet Visible Spectroscopy</td>
<td>Optical properties, size, concentration, and agglomeration state, hints at nanoparticles shape</td>
</tr>
<tr>
<td>11</td>
<td>PL Spectroscopy</td>
<td>Photoluminescence spectroscopy</td>
<td>Optical properties, relation to structural features such as defects, size, composition, etc.</td>
</tr>
<tr>
<td>12</td>
<td>DLS</td>
<td>Dynamic Light Scattering</td>
<td>Hydrodynamic size, detection of agglomeration</td>
</tr>
<tr>
<td>13</td>
<td>NTA</td>
<td>Nanoparticle Tracking Analysis</td>
<td>Nanoparticles size and their distribution</td>
</tr>
<tr>
<td>14</td>
<td>DCA</td>
<td>Direct Coupling Analysis</td>
<td>Nanoparticles size and their distribution</td>
</tr>
<tr>
<td>15</td>
<td>ICP-MS</td>
<td>Inductively Coupled Plasma Mass Spectroscopy</td>
<td>Elemental composition, size, size distribution, NP concentration</td>
</tr>
<tr>
<td>16</td>
<td>SIMS, ToF-SIMS, MALDI</td>
<td>Sputtering Ion Mass Spectroscopy</td>
<td>Chemical information on functional groups especially surface sensitivity, molecular orientation, and</td>
</tr>
<tr>
<td>No.</td>
<td>Method</td>
<td>Description</td>
<td>Measurements/Characterization</td>
</tr>
<tr>
<td>-----</td>
<td>--------</td>
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<td>--------------------------------</td>
</tr>
<tr>
<td>17</td>
<td>VSM</td>
<td>Vibrating Sample Magnetometer</td>
<td>Magnetic properties of nanomaterials</td>
</tr>
<tr>
<td>18</td>
<td>Contact Angle</td>
<td>Contact Angle</td>
<td>Determination of hydrophobic characters of thin films</td>
</tr>
<tr>
<td>19</td>
<td>FMR</td>
<td>Ferromagnetic Resonance Spectroscopy</td>
<td>Nanoparticle size and distribution, shape, crystallographic imperfections, surface composition, M value, magnetic anisotropic constant, demagnetization fields</td>
</tr>
<tr>
<td>20</td>
<td>XMCD</td>
<td>X-Ray Magnetic Circular Dichroism</td>
<td>Site symmetry and magnetic moments of transition metal ions in ferro and ferri magnetic materials element-specific</td>
</tr>
<tr>
<td>21</td>
<td>CLSM</td>
<td>Confocal Laser Scanning Microscope</td>
<td>Imaging, ultrafine morphology</td>
</tr>
<tr>
<td>22</td>
<td>BAM</td>
<td>Brewster Angle Microscope</td>
<td>Gas-liquid interface imaging</td>
</tr>
<tr>
<td>23</td>
<td>APM</td>
<td>Atomic Probe Microscopy</td>
<td>Three Dimensional Imaging</td>
</tr>
<tr>
<td>24</td>
<td>MFM</td>
<td>Magnetic Force Microscopy</td>
<td>Magnetic Material Analysis</td>
</tr>
<tr>
<td>25</td>
<td>LEED</td>
<td>Low Energy Electron Diffraction</td>
<td>Surface/Adsorbate bonding</td>
</tr>
<tr>
<td>26</td>
<td>AEM</td>
<td>Auger Electron Microscopy</td>
<td>Chemical Surface Analysis</td>
</tr>
<tr>
<td>27</td>
<td>CFM</td>
<td>Chemical Force Microscopy</td>
<td>Chemical/Surface Analysis</td>
</tr>
<tr>
<td>28</td>
<td>FIM</td>
<td>Field Ion Microscopy</td>
<td>Chemical Profile/ Atomic spacing</td>
</tr>
<tr>
<td>29</td>
<td>UPS</td>
<td>Ultraviolet Photoemission Spectroscopy</td>
<td>Surface Analysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>30</td>
<td>AAS</td>
<td>Atomic Absorption Spectroscopy</td>
<td>Chemical Analysis</td>
</tr>
<tr>
<td>31</td>
<td>ICM</td>
<td>Inductively Coupled Microscopy</td>
<td>Elemental Analysis</td>
</tr>
<tr>
<td>32</td>
<td>SANS</td>
<td>Small Angle Neutron Scattering</td>
<td>Surface Characterization</td>
</tr>
<tr>
<td>33</td>
<td>CL</td>
<td>Cathodoluminescence</td>
<td>Characteristic Emission</td>
</tr>
<tr>
<td>34</td>
<td>Nanocalorimetry</td>
<td>Nanocalorimetry</td>
<td>Latent Heat of Fusion</td>
</tr>
<tr>
<td>35</td>
<td>Sears Method</td>
<td>Sears Method</td>
<td>Colloidal size, specific surface area</td>
</tr>
<tr>
<td>36</td>
<td>FS</td>
<td>Fluorescent Spectroscopy</td>
<td>Elemental Analysis</td>
</tr>
<tr>
<td>37</td>
<td>LSPR</td>
<td>Localized Surface Plasmon Resonance</td>
<td>Nanosized particle Analysis</td>
</tr>
<tr>
<td>38</td>
<td>Rutherford Backscattering</td>
<td>Rutherford Backscattering</td>
<td>Quantitative Elemental Analysis</td>
</tr>
<tr>
<td>39</td>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
<td>NP size, size monodispersity shape, aggregation state, detect and localize quantify nanoparticles in matrices, study growth kinetics</td>
</tr>
<tr>
<td>40</td>
<td>HR-TEM</td>
<td>High-Resolution Transmission Electron Microscopy</td>
<td>All information by conventional TEM and also on the crystal structure of a single particle. It is used to distinguish between monocrystalline, polycrystalline, and amorphous</td>
</tr>
<tr>
<td>41</td>
<td>Liquid TEM</td>
<td>Liquid Transmission Electron Microscopy</td>
<td>Depict nanoparticle growth in real-time, study growth mechanism, single particle motion, and superlattice formation</td>
</tr>
<tr>
<td>42</td>
<td>Cryo-TEM</td>
<td>Cryo Transmission Electron Microscopy</td>
<td>Study complex growth mechanisms, and aggregation pathways, good for molecular biology and colloidal chemistry to avoid the presence of artifacts or destroyed samples</td>
</tr>
<tr>
<td>43</td>
<td>ED</td>
<td>Electron Diffraction</td>
<td>Crystal structure, lattice parameter, study order, and disorder transformation, long-range order parameters</td>
</tr>
<tr>
<td>44</td>
<td>STEM</td>
<td>Scanning Transmission Electron Microscopy</td>
<td>Combined with HAADF, and EDX for morphology study, crystal structure, and elemental composition, Study the atomic structure of hetero-interface</td>
</tr>
<tr>
<td>45</td>
<td>Aberration-corrected STEM, TEM</td>
<td>Aberration corrected Scanning Transmission Electron Microscopy</td>
<td>Atomic structure of NP clusters, especially bimetallic ones, as a function of composition, alloy, homogeneity, phase segregation</td>
</tr>
<tr>
<td>46</td>
<td>EELS</td>
<td>Electron Energy Loss Spectroscopy</td>
<td>Type and quantity of atoms present, chemical states of atoms, collective interaction of atoms with neighbors, bulk plasma resonance</td>
</tr>
<tr>
<td>46</td>
<td>Electron tomography</td>
<td>Electron tomography</td>
<td>Realistic 3D particle visualization, snapshots, video, and quantitative information down to atomic scale</td>
</tr>
<tr>
<td>48</td>
<td>EBSD</td>
<td>Electron Backscattered Diffraction Microscopy</td>
<td>Structure, crystal orientation, and phase of matrices in SEM. Examine microstructure, reveal texture, defects, grain morphology, deformation</td>
</tr>
<tr>
<td>49</td>
<td>AFM</td>
<td>Atomic Force Microscope</td>
<td>Nanoparticle size and shape in 3D mode, evaluate the degree of covering</td>
</tr>
</tbody>
</table>
of a surface with nanoparticle morphology, dispersion of nanoparticles in cell and other matrices/supports, precision in the lateral dimension of nanoparticles, quick examination - elemental composition

| 50 | MFM | Magnetic Force Microscopy | Standard AFM imaging together with the information of magnetic moments of single NPs. Study magnetic NPs in the interior of cells. Discriminate from non-magnetic NPs |

**Table 7. Analytical Techniques for Nanoscale Parameters**

<table>
<thead>
<tr>
<th>Entity Characterized</th>
<th>Possible Characterization Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (structural properties)</td>
<td>TEM, XRD, DLS, NTA, SAXS, HRTEM, SEM, AFM, EXAFM, FMR, DCS, ICP-MS, UV-Vis, MALDI, NMR, TRPS, EPLS, Magnetic susceptibility</td>
</tr>
<tr>
<td>Shape</td>
<td>TEM, HRTEM, AFM, EPLS, FMR, 3D-tomography</td>
</tr>
<tr>
<td>Elemental chemical composition</td>
<td>XRD, XPS, ICP-MS, ICP-OES, SEM-EDX, NMR, MFM, LEIS</td>
</tr>
<tr>
<td>Crystal structure</td>
<td>XRD, EXAFS, HRTEM, STEM, electron diffraction</td>
</tr>
<tr>
<td>Size distribution</td>
<td>DCS, DLS, SAXS, NTA, ICP-MS, FMR, DTA, TRPS, SEM, Superparamagnetic Relaxometry</td>
</tr>
<tr>
<td>Magnetic properties</td>
<td>SQUID, VSM, MFM, FMR, XMCD, Magnetic Susceptibility</td>
</tr>
<tr>
<td>Optical properties</td>
<td>UV-Visible spectroscopy, Photoluminescence spectroscopy, EELS-STEM</td>
</tr>
<tr>
<td>Detection of Nanoparticles</td>
<td>TEM, SEM, STEM, EBSD, Magnetic Susceptibility</td>
</tr>
<tr>
<td>Structural defects</td>
<td>HRTEM, EBSD</td>
</tr>
<tr>
<td>Dispersion of nanoparticles in matrices</td>
<td>SEM, AFM, TEM</td>
</tr>
<tr>
<td>3D visualization</td>
<td>3D topography, AFM, SEM</td>
</tr>
</tbody>
</table>

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2.3 UV-visible spectroscopy

UV-Visible spectrophotometric technique is the versatile technique used in many discipline of science, technology, pharmacy and veterinary medicine. Also, used to get an idea about the formation of nanomaterials, chemical analysis of noble metals and the rate of formation of nanoparticles. UV-visible spectroscopy is as shown in Fig. 4 is used for the measurement of the intensity of absorption in near-ultraviolet and visible radiation by a sample. It measures the amount of discrete wavelength of UV-Visible light that is absorbed by or transmitted through a sample in comparison to the reference or blank sample. This property is influenced by the sample composition, potentially providing qualitative and quantitative information. UV-visible radiation ranges in wavelength from 200 to 800 nm and is energetic enough to promote outer electrons in an atom to higher energy levels \([105]\). Moreover, UV-visible spectroscopy can also be used to determine the band gap of semiconductors. This band gap can be calculated by the equation 2;

\[
\Delta Eg = \frac{1240}{\lambda_{\text{max}}}
\]

As UV radiations are associated with high energy, it produces a change in the electronic energy of the molecule. Since spectroscopic techniques relies on light. The energy of electromagnetic waves is directly proportional to its frequency and inversely proportional to its wavelength. All electromagnetic waves travel with same speed if medium is same. The relationship between the energy absorbed in an electron transition and frequency \((\nu)\), wavelength \((\lambda)\), and wave number \((\tilde{\nu})\) of radiation producing the transition can be explained as given below:

<table>
<thead>
<tr>
<th>Single-particle properties</th>
<th>SP-ICP-MS, UV-Vis, RMM-MEMS, PTA, DCS, TRPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>DCS, RMM-MEMS</td>
</tr>
<tr>
<td>Agglomeration state</td>
<td>Zeta potential, DLS, DCS, UV-Visible spectroscopy, SEM, Cryo-TEM, TEM</td>
</tr>
<tr>
<td>Concentration</td>
<td>ICP-MS, UV-Visible, RMM-MEMS, PTA, DCS, TRPS</td>
</tr>
<tr>
<td>Surface charges</td>
<td>Zeta potential, EPM</td>
</tr>
<tr>
<td>Surface area, specific surface area</td>
<td>BET, liquid NMR</td>
</tr>
<tr>
<td>Ligand binding/ composition/ density/ arrangement/ mass, surface composition</td>
<td>XPS, FTIR, NMR, SIMS, FMR, TGA, SANS</td>
</tr>
<tr>
<td>Growth kinetics</td>
<td>SAXS, NMR, TEM, cryo-TEM, liquid-TEM</td>
</tr>
<tr>
<td>Chemical state -oxidation state</td>
<td>XAS, EELS, XPS, Mossbauer</td>
</tr>
</tbody>
</table>
\[ \Delta E = h \nu = \frac{hc}{\lambda} = hcv \]  

(2)

where \( h \) is the plank’s constant, \( c \) is the velocity of light in air or vacuum and its value is \( 3 \times 10^8 \text{m/s} \) [106]. This is the fastest speed in nature. Nothing can travel faster than velocity of light. If anything found to travel faster than speed of light, today’s knowledge of physics would be highly affected and \( \Delta E \) is the energy absorbed in the electronic transition in a molecule from a low energy state (ground state) to a high energy state (excited state).

Meanwhile, the amount of energy absorbed depends upon the energy difference between the two states i.e. ground state and excited state; the smaller the difference, thus, in smaller difference less energy would be required for promotion of electron from ground state to excited state and ultimately the longer the wavelength of absorption takes place. The position of an absorption band corresponds to the wavelength of radiation whose energy is equal to the required for an electronic transition. The intensity of absorption can be derived from the Beer-Lambert law [107]:

\[
- \frac{dl}{dx} = klc
\]

\[
- \frac{dl}{dx} = klc
\]

\[
- \frac{dl}{l} = kdc
\]

Integrating the above equation, we get,

\[
- \int_{l_0}^{l} \frac{dl}{l} = k c \int_{x_0}^{x} dx
\]

\[
- \log_e \frac{l}{l_0} = k c x
\]

\[
\log_e \frac{l}{l_0} = -k c x
\]

\[
I = I_0 e^{-kcx}
\]

(3)

Equation 3 is famous and well known as Beer–Lambert’s equation which provides the direct relationship between energy, frequency, and wavelength of radiation. Generally, the metallic nanomaterials exhibit characteristic colors having particular metallic lusters. The absorption of electromagnetic radiation by metallic nanoparticles originates from the coherent oscillation of valence band electrons induced by interaction with an electromagnetic field. These resonances are known as surface plasmon occurs only with nanomaterials. The noble metal colloidal solutions are showing the phenomenon of Surface Plasmon Resonance; thus UV-visible spectroscopy is a powerful tool for its characterization [108].
Fig 4. Schematic diagram for the experimental setup of UV-Vis spectroscopy (Lic: CC BY 4.0 Ref-105)

2.4 Nuclear Magnetic Resonance Spectroscopy (NMR):

NMR spectroscopy is a type of spectroscopic techniques in which radio frequency waves induce transition between magnetic energy level of nuclei of a molecule. The magnetic energy levels are created by keeping the molecule in the magnetic field. In absence of magnetic field, the spin state of nuclei are degenerate. When a magnetic field is applied, the separate levels and radiofrequency radiations can cause transition between these two energy levels. Among all types of electromagnetic radiations, radio-waves are of lowest energy. The frequency of radio-waves varies from $10^7$ to $10^8$ cycles per seconds. Thus, the amount of energy associated with radio waves is very less. Thus the radio waves are unable to vibrate, rotate or excite an atom or a molecule. But the amount of energy associated with radio waves are enough to affect nuclear spin of atoms or molecules.

Two types of NMR spectroscopy are commonly used today i.e. $^1$H and $^{13}$C spectroscopy. When a compound is subjected to PMR spectroscopy we obtain three types of information: (1) The relationship between the number of signals in the spectrum and number of different kinds of hydrogen atoms in the molecule. Thus, one can know the different kinds of environments of the hydrogen atoms in the molecule. (2) The principle signal may get split into smaller peaks i.e. spin-spin splitting may be observed. The types splitting pattern observed depends on the number of neighboring non-equivalent protons. For many simple compounds, one can predict splitting pattern with the n+1 rule, where n is the number of neighboring protons. Spin-spin splitting thus help in deducing molecular structure. (3) Spacing between the peaks is labelled J which gives the units of cycle per second. J is coupling constant between two protons and J values give further information on molecular structure and stereo-chemical features. Thus, e.g. J value between adjacent axial hydrogen in a cyclohexane is 10-13 Hz, while J between axial and equatorial or between two equatorial hydrogens is 3-5Hz.

Each atom contains proton, neutrons and electron. In case of Hydrogen atom there is no neutron. The unpaired proton can act as tiny magnet. This proton acts as tiny magnet due to spin movement.
When an atom is having spin quantum number $I=0$, then it is NMR active for example $^{12}\text{C}$. When the atom is having $I \geq 0$, are NMR active. Thus, $^{13}\text{O}$, $^{31}\text{P}$, $^{13}\text{N}$ etc., are also NMR active. These all can be used in NMR analysis. But generally $^{13}\text{C}$ and $^{1}\text{H}$ are used as NMR active materials. This is because there is very little abundance of other elements in the compounds. Hence generally hydrogen and carbon are used in the NMR spectroscopy. In case of Hydrogen $I=\frac{1}{2}$ and is NMR active. When an external magnetic field is applied then the protons get aligned. Each proton is having different capacity to align in the presence of external magnetic field.

2.4.1 Instrumentation: A high resolution NMR spectroscopy is having complex collection of electronic equipment. Here we have to deal with the intense magnetic field and requires precise controlled power supply as well as frequencies. It is worth to note that most of the power required by the instrument is dissipated in the form of heat and very small amount of it obtained as a signal which has to be amplified by a complex electronic system. The instrument consists of permanent magnet, radio frequency generator, radio frequency detector, sample holder and magnetic coils are as depicted in Fig.5.

(a) Permanent Magnet: This is an important component of NMR spectroscopy. The permanent magnet should be such that it should provide homogeneous magnetic field i.e. strength and direction of magnetic field should remain uniform in all points. Also the strength of the field should be very high as much as 20,000 gauss. The chemical shift is directly proportional to the field strength. Permanent magnets can provide fields that are sufficiently constant provided that they are thermostatically maintained carefully. The conventional electromagnets are not less stable but the expected stability can be obtained by producing ancillary stabilizing devices. The electromagnets containing superconducting solenoids operating in inert gas liquid helium crystals are very stable. The electromagnets interact much less with external magnetic effect as compared with permanent magnets. The homogeneous magnetic field can be developed both by permanent magnets and electromagnets by carefully machining and alignment of pole faces. The current shimes are used to produce field gradient in various directions so as to cancel out gradient inherent in the main field. The magnet used in NMR spectroscopy should be such that it can produce very strong magnetic field. It is also important that magnetic field be constant over long period of time. For this reason, adequate temperature control is necessary to maintain the constant physical dimension of the magnet. The adequate temperature control is necessary to maintain. For this, the magnet system is usually thermostatically controlled, also it is advantageous to control the temperature of room in which the instrument is placed.

(b) Radio frequency generator: Radio frequency is essential for operating the NMR instrument. Hence in order to generate radio frequency, radio frequency oscillators are used. In order to achieve maximum interaction of the radio frequency waves with the sample under study, the coil of oscillator is wound around the sample container. The oscillator irradiates the sample with radio
frequency radiation. The oscillator coil is wound perpendicular to the applied magnetic field. This is done so that the applied radio frequency field should not change the effective magnetic field in the process of irradiation.

(c) **Radio frequency receiver:** As we have seen the radio frequency is exposed on the sample. When the sample is irradiated with radio frequency two phenomenon i.e. absorption and desorption are taking place simultaneously. The observation of either absorption or desorption will enable the resonance frequency to be determined. The interpretation of absorption spectrum is easy as compared with the absorption of desorption spectrum.

(d) **Sample holder:** The NMR spectroscope requires a sample holder. The sample holder should be chemically inert, durable and transparent to radio frequency radiations. Generally, glass tubes are used which can be easily cleaned, economical, sturdy etc. The glass tubes are generally made up of borosilicate glass of 8.5 cm long and 0.3 cm diameter.

(e) **Magnetic coil:** This is another very important component of NMR spectroscopy. There is relationship between the resonance frequency of the nucleus and the strength of magnetic field in which the sample is placed. The relationship can be expressed as below,

\[ \Gamma = \text{constant} \times H_0 \]

For any nucleus to resonant the precession frequency of the nucleus must be equal to the applied radio frequency of radiation. Thus, we note that if Ho is kept constant, the precession frequency also remains fixed. It is not easy to change magnetic field of a large stable magnet. But it is possible to overcome by superimposing a small variable magnetic field on the main field. This is done by using pair of Helmhotlz in the pole faces of permanent magnet. These coil induce the magnetic field that can be varied by varying the current flowing through them. The small magnetic field is produced in the same direction as the main field is added. The sample is exposed to both fields, which appear as one of the molecules.
2.5 X-Ray Diffraction (XRD):

In material research researchers and scientists have to determine the chemical composition and crystalline constitution of materials. To date x-ray diffraction is available non-destructive and accurate laboratory technique which can provide information about the chemical composition, crystal structure, crystal orientation, crystallite size, lattice strain, phase composition, preferred orientation of powder, solid and liquid samples and layer thickness. Several materials are made up of tiny crystallites. The
chemical composition and structure of these tiny crystallites are called phase. The materials can be single phase or multiple phase mixture. The materials can contain crystallite and non-crystalline compositions at the same time. When the sample is exposed to x-ray diffraction pattern, different crystallite structures give different x-ray pattern. Phase identification can be performed by comparing the diffraction pattern. Today, the most comprehensive data base is maintained by ICDD. X-rays were discovered in 1895 by German physicist Wilhelm Conrad Rontgen. They were called X-rays because their nature was unknown at that time. X-rays are invisible, highly penetrating electromagnetic radiations of much shorter wavelengths than visible light. X-rays are electromagnetic radiations with a wavelength of the order of $10^{-10}$ m. Van Lave demonstrated in 1912 that X-rays could be diffracted by crystal. Later, in year 1935 Le Galley first constructed an X-ray powder diffractometer. After this invention, metallurgists and mineralogists use X-ray powder diffractometers primarily to study structural imperfections. X-rays scatter or fluorescence as a result of interaction with electrons in the materials to which they are irradiated. This phenomenon can provide information about the detail structure of an object and also provides information about its constituents. X-ray diffraction study has been widely in metallurgy and material sciences.

X-ray powder diffraction is the rapid analytical technique primarily used for identification of the proper phase and crystalline material. The analyzed material is finely ground, homogenized and average bulk composition is determined. They are typically generated by bombarding a metal with high-energy electrons. The high-energy electrons must penetrate through the outer electron shells and interacts with the inner shell. If more than a critical amount of energy is transferred to an inner-shell electron, that electron is ejected i.e. it escapes the attractive field of the nucleus, leaving the hole in the inner shell and generating an ionized atom. The ionized atom can return almost to its lowest energy (ground state) by filling in the missing electron with one from the outer shells. It is this transmission that is accompanied by the emission of an X-ray or Auger electron. It is the transition which is accompanied by the emission of an X-Ray or Auger electron.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Target Metal</th>
<th>$\lambda$ of Ka Radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mo</td>
<td>0.71Å°</td>
</tr>
<tr>
<td>2</td>
<td>Cu</td>
<td>1.54Å°</td>
</tr>
<tr>
<td>3</td>
<td>Co</td>
<td>1.79Å°</td>
</tr>
<tr>
<td>4</td>
<td>Fe</td>
<td>1.94Å°</td>
</tr>
<tr>
<td>5</td>
<td>Cr</td>
<td>2.29Å°</td>
</tr>
</tbody>
</table>
The principle of working of XRD ‘the elastic scattering of the X-rays from structure that have long range order’. The x-rays get diffracted by a crystal because the wavelength of x-rays is similar to the inter atomic spacing in the crystal. X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. British physicist Sir W.H. Braggs and W.L. Braggs developed a theory in 1913 to explain why cleavage faces of crystals appear to reflect X-ray only at a certain angle of the incident (θ). This equation is called Bragg’s law and is given as,

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

where, \( d \) is the distance between the two atomic layers in crystal and \( \lambda \) is the wavelength of the incident X-ray beam, and \( n \) is an integer that indicates the order of reflection. This law relates wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through the range of 2θ angle, all possible diffraction directions of the lattice should be attained due to random orientation of the powdered material. This observation is an example of X-Ray diffraction and was direct evidence for the periodic atomic structure of crystals postulated for several centuries. Although Bragg’s law was used to explain the interference pattern of X-Ray scattered by crystals, diffraction has been developed to study the structure of all states of matters with any beam of ions, electrons, neutrons and protons with the wavelength similar to the distance between atomic and molecular structures of interest. Bragg’s law is represented in Fig. 2.

![Bragg’s law for X-ray diffraction](image)

**Fig.2:** Bragg’s law for X-ray diffraction

A crystal may be defined as a collection of atoms arranged in a pattern that is periodic in 3D. Crystals are necessarily solid, but all solids are not crystalline in nature. In a perfect single crystal all atoms in the crystal are related either through translational symmetry as point symmetry. Polycrystalline materials are made up of great number of tiny crystals. In powder diffraction method, we can get information, from peak intensities unit cell contents, point symmetry and from peak shapes with width crystalline size, non-uniform micro-strain, extended defects. A perfect crystal would extend in all directions to indefinitely, so we can say that no crystal is perfect due to its finite size. This deviation from perfect crystallinity leads to a broadening of the diffraction peaks. Crystalline size is a measure of...
the size of coherently diffraction domain. Powder XRD is an important characterization tool to determine the structure of nanomaterials. Their crystalline nature could be identified by the XRD pattern. As shown in Fig. 3, the sample to be analyzed is generally in powder form, consisting of randomly oriented fine grains of crystalline material. XRD offers unparalleled accuracy in the measurement of atomic spacing and is the technique of choice for determining strain states in thin film. The intensities measured with x-ray can provide correct information on the atomic arrangements at interfaces. The position and intensities of the peak are used to identify the underlying atomic arrangements in the sample. The x-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate and directed towards the sample. The interaction of the incident rays with the sample produces constructive interference. In the powder X-ray diffraction method, we can get information from peak intensities and their position about the unit cell content, and point symmetry.

![Fig. 3: Bragg-Brentano para-focusing geometry of XRD in reflection mode](image)

### 2.5.1 Working Mechanism of XRD Instrument:

The principle component of X-ray instrument comprises of mainly three parts viz. X-ray source, a sample holder and the X-ray detector. X-rays produced by either X-ray tube which or synchrotron radiations and the source illuminate the sample. When the focused X-rays are electron beams are accelerated across high voltage field, generation of X. This is then diffracted by the sample phase and incident on the detector. The diffraction angle can be changed by rotating tube or sample and detector. The intensity of diffracted x-ray is measured and diffraction data is recorded. In 1918, Scherrer first observed that small crystalline size gives rise to line broadening, and based on this observation he derived the well-known equation called the Scherrer formula which relates the crystalline size to the line broadening and is given as

$$D = \frac{k \lambda}{\beta \cos \theta}$$

The typical example of application of X-rays includes the followings:
(1) Observation of the structural change of material under reactions of manufacturing processes or the environment.
(2) Obtaining high accuracy information on material structure unobtainable by conventional methods.
(3) Obtaining structural information on materials in regions micrometers to nanometers from surface to phase boundary.

2.5.2 Applications of XRD:

Many researchers from industrial sectors, medicine, pharmacy and academics rely on X-ray diffraction technique for determination of structure and composition of unknown materials. Hence, we would like to mention that just very similar to the use of NMR as an organic chemist uses for structural determination of an organic compound, the researchers working in material science uses XRD diffraction pattern in order to elucidate the structure and composition of crystalline material under study. Thus, we realize that x-ray diffraction can be used for qualitative as well as quantitative analysis of pure substance and the mixtures. The commonly used method for phase analysis is X-ray powder diffractometer. The applications of XRD can be enlisted as below:

- Identification of fine grained minerals such as clays and mixed layer clays that cannot be easily determined through optical instruments.
- Characterization of crystalline materials.
- Determination of purity of the sample.
- Determination of modal amounts of minerals.
- The thin films can be characterized.
- Determination of the thickness, roughness and density of the thin film using glancing incidence X-ray reflectivity measurement.
- Measurement of super-lattice in multilayer epitaxial; structure.
- Determination of dislocation density and quality of the film by rocking curve measurement.
- Determining lattice mismatch between film and substrate and to inferring stress and strain.

2.5.3 Strength and Limitations of XRD Technique:

a) Strength of the Technique:

- XRD units are widely available and easy to operate. It does not require very elaborate training.
- This is non-destructive method of analysis. This means the sample can be further used for other purposes.
- Minimal sample preparation is required.
- Powerful and rapid technique for identification of unknown material.
- Data interpretation is simple and straightforward.
- In most cases it provides an unambiguous mineral determination.
• This a relatively rapid technique for structure and composition determination

b) Limitations:

• This technique requires tenths of a gram of sample which must be ground into fine powder.
• There must be access to standard reference files of inorganic compounds. In absence of standard reference file, comparison of the XRD pattern would not be possible.
• Homogeneous and single phase material is best for identification of an unknown.
• In case of mixture of the materials, the materials having at least 2% amount can only be determined.
• The unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.

2.6 Fourier Transform Infrared Spectroscopy

Fourier transform spectroscopy is a technique that uses interference of light rather than dispersion of light and measures the spectrum of the sample under study. This is a common and popular spectroscopic technique used to identify the various functional groups presents in organic compounds. The application of FT-IR can be extended towards material science, where the reduction process of metal ions can be successfully recognized using FT-IR. The spectroscopy in the middle IR region is the very useful for the identification of organic compounds. This technique is widely used in organic chemistry due to the complex structure which gives numerous maxima and minima. Modern spectrometers working in the infrared region generally use the Fourier transform technique for spectral detection and analysis. The atoms in a molecule do not remain in a fixed relative position and keep vibrating about their mean positions. Due to this vibrational motion, if there is a periodic alternation in the dipole moment, such modes of vibration are IR active. The IR region of the electromagnetic spectrum ranges from 100µm to 1µm wavelength. The vibrating molecules absorb energy only from those radiations with which they can coherently interact i.e. radiations of their oscillation frequency.

Thus each functional group has its specific vibrational frequencies which are very sensitive to its chemical environment and neighboring species. The molecular structure of a compound can be guessed from the appearance or nonappearance of certain vibrational frequencies. The to and fro movement of particle about its mean position is called as vibrational frequency. The principle of vibrational spectroscopy or infra-red spectroscopy is that molecules absorb specific frequencies of light matches corresponding structure of molecule. The energies are dependent on the vibronic coupling, mass corresponding to its atoms, and the shape of the molecular surface. The vibrational frequency is given as,
\[ \nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \]  

(6)

where \( k \) is force constant and \( \mu \) is reduced mass.

The key concept in FTIR is the use of a mathematical operation called Fourier transformation which converts a signal in the time domain to the frequency domain. The frequency-domain transformation speeds up the estimation of the IR spectrum of the signal. The time required here is 1 sec or less vs 10-15 minutes for the normal system. The reduction in time allows for taking several sample scans and thereby improving the resolution and quality of the FTIR imaging. In FTIR spectroscopy, we are using the term wave-number which is reciprocal to wavelength.

The choice of a capping agent may also affect the FTIR image. When the capping molecules are adsorbed on the nanoparticle surface, their vibration modes will change. Hence the vibrational frequency characteristics of the functional group will be either shifted and the extent of such shifting depends upon the nature and strength of interactions\(^{69}\).

FTIR Instrumentation: Infrared spectrometer has similar components to those of ultraviolet and visible instruments. The essential components of an infrared spectrometer are

2.6.1 Light source: A Globar is used as a source of light in FT-IR. The material preferred for Globar is silicon carbide that is shaped as rods or aches of different sizes. The light source for a FTIR spectrometer is typically a broadband emitter, such as mid-IR ceramic source, Mid and near IR used tungsten-halogen source lamp and far IR mercury lamp that provides good intensity over the wide range.

2.6.2 Monochromatic and optical material: The FT-IR spectroscopy uses reflection optics to observe and focus infrared light onto the sample. The important part in the selection of material as an optical material depends on degree of transparency and refractive index. The most common optical material used in FT-IR is calcium fluoride, fused silica, germanium, magnesium fluoride, potassium bromide, sapphire, sodium chloride, zinc selenide, zinc sulfide etc.

2.6.3 Sample holder: There are different types of sample holders for FT-IR spectroscopy. Herein we will discuss various types of sample holders in short:

(a) Universal holder: This is a versatile all round shape holder for transmission analysis. This type of sample holder can be used for films, pellets, windows, mulls etc.

(b) K-holder: This is another type of sample holder. This sample holder can be used for liquid samples, pellets etc. Base and positioning mount can be up and down in order to position the sample correctly according to its specific size and form. The standard size back plate fits in all types of spectrophotometers.
(c) **EZ-Clip:** This is basic and easy to use sample holder to effortlessly hold windows, pellets and films in place. The spring loaded clips hold samples firmly at proper place. The standard size back plates fit in all spectrophotometers.

(d) **Quick holder:** The sample holder is designed to hold collars for 1,3,7 and 13mm KBr pellets. The magnetic back plate holds the sample in place. An opening at the top end of the holder is used for 13mm pellets.

2.6.4 **Detector:** The detectors can be classified in three different categories such as thermal detector, pyroelectric detectors and photo-conducting detectors. Finally, the response of the detector is measured using some indicating instrument.

2.6.5 **Modern Infrared spectrometers:** They are working in the infrared region generally use the Fourier transform technique for spectral detection and analysis. The essential part of an FTIR instrument is Michelson’s interferometer, which consists of a static and a movable mirror as shown in Fig. 4. Michelson’s interferometer was constructed by Albert A Michelson in 1887. The experimentation of Michelson’s interferometer gave an idea to the scientific world that all waves needs a medium for its propagation. Then question was raised how light waves travel from Sun to earth. To answer this, it was assumed that there is the hypothetic medium present between earth and sun. This hypothetical medium is termed as luminiferous aether or ether medium. Principle of working of Michelson’s interferometer: A Michelson’s interferometer works by using a half silvered mirror to split an incoming wave into two equal waves. The waves after splitting are sent in different perpendicular directions. These two waves after travelling a particular distance, encounter a plane mirror and is sent back to the silver mirror, where the two light waves are then directed to an observation screen or detector and the two light waves half recombine and produce an interference pattern. This interference pattern and how it changes during an experiment, can be analyzed to make the measurements in different fields.

The source is generally a Nernst filament consisting of a spindle of rare-earth oxides. The beam is then guided and made to pass through a beam splitter (a part of the interferometer, a component that reflects about half of the radiation that hits it and transmits the rest). The beam thus divided into 2 parts recombines again either constructively or destructively depending on the path difference created by the movable mirror. An actual signal thus consists of radiation spanning a large number of wave numbers and the total intensity is thus integrated from the individual contributions75.
2.7 Microscopic Techniques

Animal eye is an optical instrument. It translates photons into electrical signal that human learn and recognize as light and colour. Naked eye is the practice of engaging in visual perception unaided by a magnifying or light collecting optical instrument such as telescope or microscope. For the observation of a nearby object, without magnifying glass or microscope, the size of the object depends upon viewing distance. Under the condition of normal light or brightness the angular size recognized by naked eye is around 1 arc minutes = 1/60 degrees = 0.003 radians. If one is desirous to visualize objects smaller than this, some external aid is required. Microscope is the instrument used for magnification and resolution of the smaller objects. Microscopy is the science of investing small objects and their structure using such an instruments. Microscopy means invisible to eye unless aided by the instrument microscope. A significant contribution in the area of microscope is devoted by Dutch Physicist Antonie Philips van Leeuwenhoek. Later on much advancement has taken place in the area of microscopes.

Microscopy is the technical field of using a microscope to view samples and objects that cannot be seen with the unaided eye. There are three well-known branches of microscopy; optical, electron, and scanning probe microscopy. Optical and electron microscopy involve the diffraction, reflection, and refraction of electromagnetic radiation/ electron beam interacting with the specimen and the subsequent collection of this scattered radiation or another signal to create an image. This process may be carried out by wide-field irradiation of the sample (e.g. Transmission Electron Microscope) or by scanning a fine beam over the sample (e.g. Scanning Electron Microscope). The developmental scenario in the microscopy can be summarized in Table 9 as below:

**Table 9.** Chronological Developmental Scenario in Microscopy
<table>
<thead>
<tr>
<th>Year</th>
<th>Major Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>167 BCE</td>
<td>The Chinese use water made of a lens and water-filled tube to visualize the unseen.</td>
</tr>
<tr>
<td>1000 AD</td>
<td>Reading stone. The first vision aid called the reading stone was invented. It is a glass sphere placed on the top of the text which magnifies readability.</td>
</tr>
<tr>
<td>1284</td>
<td>The first eyeglass was invented. Salvino Darmate invented the first wearable glass.</td>
</tr>
<tr>
<td>14th century</td>
<td>Spectacles were first made in Italy</td>
</tr>
<tr>
<td>1590</td>
<td>Dutch spectacle maker Hans Jansen and his son Zacharias Jansen were claimed by later writers to have invented the compound microscope</td>
</tr>
<tr>
<td>1609</td>
<td>Galileo Galilei developed a compound microscope with a convex and a concave lens.</td>
</tr>
<tr>
<td>1619</td>
<td>Cornelius Drebbel presents, in London a compound microscope with two convex lens.</td>
</tr>
<tr>
<td>1620</td>
<td>Christian Huygens, another Dutchman developed a simple 2 lens ocular system that was chromatically corrected.</td>
</tr>
<tr>
<td>1665</td>
<td>Robert Hooke published Micrographia a collection of biological micrographs. He coins the word cell for the structures he discovers in cork bark</td>
</tr>
<tr>
<td>1674</td>
<td>Anton van Leeuwenhoek improved a simple microscope for visualizing biological objects. He was the first person to visualize bacteria.</td>
</tr>
<tr>
<td>1830</td>
<td>Joseph Jackson Lister discovered that using weak lenses together at various distances provided clear magnification.</td>
</tr>
<tr>
<td>1878</td>
<td>Ernest Abbe developed a mathematical theory linking resolution to light wavelength.</td>
</tr>
<tr>
<td>1903</td>
<td>Richard Zsimondy invented the ultramicroscope, which allows for observation of specimens below the wavelength of light.</td>
</tr>
<tr>
<td>1931</td>
<td>Ernest Ruska started to build the first electron microscope. It is a Transmission Electron Microscope.</td>
</tr>
<tr>
<td>Year</td>
<td>Event</td>
</tr>
<tr>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>1932</td>
<td>Transparent biological materials were studied for the first time using Frits Xernike’s invention of the phase-contrast microscope.</td>
</tr>
<tr>
<td>1938</td>
<td>James Hillier built another TEM</td>
</tr>
<tr>
<td>1953</td>
<td>Zernaik, a professor of theoretical Physics was awarded the Nobel prize for the invention of the phase-contrast microscope</td>
</tr>
<tr>
<td>1955</td>
<td>George Nomarski, professor of microscopy published the basis of differential interference contrast microscopy</td>
</tr>
<tr>
<td>1981</td>
<td>Gerd Binning, Qutar, and Gerber invented Atomic Force Microscopy</td>
</tr>
<tr>
<td>1988</td>
<td>Kingo Itaya invented the Electrochemical Scanning Microscope</td>
</tr>
<tr>
<td>1991</td>
<td>Kelvin, probe the force microscope invented</td>
</tr>
</tbody>
</table>

**a) Resolving power**

The resolving power of an optical instrument is its ability to differentiate between two adjacent lines or points. The greater is the resolving power, the smaller is the minimum distance between two lines or points that can still be distinguished. The basic limitation of a light microscope is its resolving power. Mere magnification of objects without the ability to distinguish structural details is not beneficial. The largest magnification produced by a microscope may not be most useful because the image obtained may be unclear or fuzzy. Therefore, for better clarity high-resolution image is desired. The resolving power of a microscope is a function of the wavelength of light used, refractive index and the numerical aperture or angular aperture (NA) of the lens system.

**b) Objective Numerical Aperture:** The numerical aperture of a microscope is a measure of its ability to gather light and resolve fine specimen detail at a fixed object distance. The numerical aperture of the microscope is given below,

\[ NA = n \sin \theta \]  

where \( n \) is the refractive index and \( \theta \) is the one-half of the angular aperture \( A \). Apart from above mentioned important factors the resolving power of an optical instrument depends upon type of specimen, coherence of illumination, degree of aberration correction and other factors such as contrast enhancing methodology either in optical system of the microscope or in specimen itself. The microscope can be broadly classified as under:

**2.7.1 Optical Microscope:** This type of microscope is the oldest design of microscopes and was probably invented during 17 centuries in Europe. Such type of microscope contains one or more lenses producing an enlarged image of the sample placed in focal plane. This type of microscope is the simplest form of microscopes which contains mainly eye pieces (ocular lens), objective (resolve or resolving
noise), focus knob, light source, condenser etc. Examples: Simple Microscope, Compound Microscope, Phase Contrast Microscope and Confocal Microscope.

2.7.2 Electron Microscope: Such type of microscope uses beam of accelerated electrons as a source of illumination. The wavelength of an electron is 100,000 times shorter than the visible photons. Consequently, electron microscopes are having higher resolving power than optical microscopes.

2.7.3 X-Ray Microscope: Such type of microscope uses electromagnetic radiations in the soft X-Ray band to produce magnified image of the object. The resolution of X-Ray microscopy lies in between optical microscope and electron microscope. The main advantage of X-Ray microscope is the visualization of biological sample in their native state.

2.7.4 Probe Microscope: Scanning Probe microscope is another type of microscope which forms images on the surface using a physical probe that scans the specimen. Examples: Scanning Tunneling Microscope, Atomic Force Microscope.

2.7.5 Bright Field Microscopy: In bright field microscopy, the microscopic field is brightly lightened. The optical part of the typical bright field microscope and the path light rays follow to produce enlargement or magnification. Generally, microscope of any type produces a uniform magnification of 1000X to 2000X. At magnification greater than 2000X, the image becomes fuzzy.

2.7.6 Dark Field Microscopy: The effect produced by dark field technique is that of a dark background against which objects are brilliantly illuminated. This is accompanied by equipping the light microscope with a special type of condenser that transmits a hollow cone of light from the source of illumination. Most of light directed through condenser does not enter the objective, the field is essentially dark. However, some of light rays will be scattered if transparent medium contains objectives. In this case will appear bright in an otherwise dark microscope.

2.7.7 Fluorescence Microscope: Many chemical substances absorb light. After absorbing light of a particular wavelength and energy. Some substances will emit light of a larger wavelength and lesser energy content. Such substances are called as florescent and the phenomenon is known as fluorescence microscopy.

2.7.8 Phase Contrast Microscope: This technique is based on the fact that light passing through one material and into other material is slightly different refractive index will undergo a change in phase. This difference in phase or wave-front irregularities are translated into variations in brightness of the structure and hence are detective to the eyes. With phase contrast microscopy, it is possible to reveal difference in cells and their structures not describe by other microscopic material. In order to overcome the limitations, set by the diffraction limit of visible light and also to image materials in label-free conditions, other microscopes have been designed which we use waves other than light.

2.7.9 Electron Microscopy
As we have discussed in earlier parts that the structural features of nanomaterials can be revealed up to certain extent by spectroscopic techniques. But the correct and accurate structure cannot be visualized through spectroscopic techniques. But it is the only microscopic technique that allows direct imaging of the particles. Among all types of microscopes, the different types of electron microscopes are most advanced techniques for imaging. In biological specimen analysis and material characterization today electron microscope is widely used. The two types of electron microscope i.e. Scanning Electron Microscope abbreviated as SEM and Transmission Electron Microscope abbreviated as TEM are most popular among the researchers working in material science and engineering. Today scanning electron microscopy is a versatile technique used in many industries as well as in research and development laboratories. In the early 1930s, many scientists and engineers realized that they have reached the theoretical limit of resolving power of an optical microscope. To see the finer details of objects such as biological cells, scientists started to develop a new type of microscope that make use of fast-moving electrons instead of light. Microscopes that make use of fast-moving electrons are known as electron microscope.

To use the microscope efficiently and with minimal frustration, we need to understand the basic idea and principle of microscopy i.e. magnification, resolution, numerical apertures, focusing and illumination. The theory behind the electron microscope is given here. The electron microscope is the microscope that uses electrons as the source of illumination. They use electron optics that are analogous to the glass lenses of the optical microscope. It is a special type of microscope having high resolution of images, and can magnify the objects in the nano meter region. The increase in kinetic energy results in a decrease in de Broglie’s wavelength of electrons. The idea is to direct a focused beam of electrons towards a small part of an object in a vacuum and detect signals generated due to the interaction of electrons concerning the object. Image can be generated depending on contrast in the magnitude of signals obtained when a beam of focused electron scans the object. An electron microscope can achieve better resolution and greater depth of focus. The scanning electron microscope uses electrons with energies of a few thousand electron volts (eV). The de Broglie wavelength of an electron is given by,

$$\lambda = \frac{h}{p}$$

Where h is Plank’s constant, and p is the momentum of the electron. Thus electron microscopes would be able to achieve much better resolution than ordinary optical microscopes.

Fast-moving electrons have very high kinetic energy and thus have a very short wavelength. Therefore, they are capable of very high resolution if that wavelength can be used in an appropriately designed instrument such as an electron microscope. The resolving power of an electron microscope is given by the following equation,
\[ d = \frac{0.61 \lambda}{NA} \] (9)

Where \( d \) is the smallest distance between two points that can be seen as distinguishable, \( \lambda \) is the wavelength and \( NA \) is the numerical aperture.

Working Principle of Electron Microscope: The electron microscope uses the signals arising from the interaction of an electron beam with the sample to obtain information about structure, morphology and composition.

- The electron gun can generate the electrons.
- Two set of condenser lenses focus the electron beam on the specimen and then into the tight beam.
- The specimen to be examined is made very thin at least 200 times thinner than the optical microscope.
- The electron beam coming out of the specimen passes to the objective lenses which has high power and forms the intermediate magnified image.
- The ocular lenses then produce the final further magnified images.
- The denser region in the specimen scatter more electrons and therefore appear darker in the image since fewer electros strike that area of the screen.

In general, the resolving power of an ordinary microscope is about 1 nm. This number is larger than the de Broglie wavelength because instrument geometry and electron scattering in the specimen are factors that influence the resolving power. The electron microscope is generally classified as Scanning Electron Microscope (SEM) and transmission electron microscopy (TEM). A comparison in terms of resolving power shows that the scanning electron microscope is somewhat intermediate between the optical microscope and transmission electron microscope. A significant advantage of using SEM compared to TEM is that the former can create an image of the surface of bulk samples with a large depth of focus. This large depth of focus also allows SEM to be used at low magnification instead of an optical microscope.

2.8 Scanning Electron Microscopy

Scanning Electron Microscope has many advantage over traditional microscope. SEM has a large depth of field, which allows more of the specimen to be in focus at one time. SEM is shown in Fig. 5. This is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with the atoms in the sample, producing various signals that can be detected and that contain information about the sample’s surface topography and composition. The electron beam scans in a raster scan pattern, and the beam’s position is combined with
the detected signal to produce an image. The SEM has much larger resolution, so closely spaced specimen samples can be magnified at much larger levels.

Scanning electron microscopy is extremely useful for direct observations of the surface because they offer better resolution and depth of field than optical microscopes. The SEM shows very detailed 3-D images at a much higher magnification than is possible with a light microscope. The image created without light waves is rendered black and white. In a very brief way, SEM works under a vacuum in a column where an electron gun emits a beam of high-energy electrons. The two major components of SEM are the electron column and control console. The electron column consists of an electron gun and two or more electron lenses, which influence the path of electrons traveling down an evacuated tube. Scanning Electron Microscopy is extremely useful for direct observation of surface because they offer better resolution and depth of field than optical microscope. The two major component of SEM are electron column and control console. The electron column consists of an electron gun and two or more electron lenses, which influences the path of electron travelling down an evacuated tube.

Fig. 5: Schematic showing the working of a Scanning Electron Microscope.

2.8.1 Instrumentation: A SEM consists of an electron gun that produces the electrons, an anode at high voltage that accelerates the electrons, a system of electromagnetic lenses that focuses the beam of electrons onto the samples, and scanning coils that facilitate the scanning of the electron beam over the sample surface, the sample chamber where the sample is located, and detectors that measure the signals
generated due to the interaction of the electrons with sample and this is shown in Fig. 6. All these components are housed within the vacuum chamber.

**Fig. 6:** An illustration showing the possible effects after sample-electron beam interaction

There are two types of electron source or electron gun; the first thermionic and the second field emission guns. A thermionic electron relies on electrons emitted from heated wire or a filament. The filament is usually a bent tungsten wire that functions as a cathode. The bent portion is heated up when an electric current passes through the filament. The outer orbital electrons of the tungsten atoms are emitted when they gain sufficient thermal energy to overcome the energy barrier that prohibits the electrons from escaping. The higher the temperature more is the number of electrons emitted. Tungsten is usually chosen as filament because it can withstand high temperatures without melting. However, thermionic electron guns have relatively low brightness. Whereas the field emission electron gun relies on electrons emitted from a sharp tip upon the application of a high electric field. It does not involve the heating of a filament. Instead, when a high electric field is applied to the tip, quantum mechanical tunneling takes place. Typically, the field emission gun has two anodes. The first anode (at 0-5 KV) serves to extract the electrons from the tip while the second anode (at 1-50KV) serves to accelerate the electrons and this determines the energy of electrons traveling down the column of SEM. The field emission gun has a higher brightness.

As electrons are streaming out of electron guns, they form a spray pattern. To control the profile of this electron beam into a finely adjusted focused beam, electromagnetic lenses are used. When an electron with charge \( q \) and velocity \( v \) travels in a region with a magnetic field \( B \) it experiences a force given by,

\[
F = qV \times B
\]  

(10)
Here it should be noted that since the direction of force acting is perpendicular to the direction of the velocity, therefore the Lorentz force acting on the electrons has no effect on the speed of electrons. The only effect the magnetic field has is on the electron which changes the direction of motion of electrons.

The magnetic profile generated by a typical electromagnet used in SEM is highly non-uniform. The magnetic field of the electromagnetic lens can be considered to be made up of two independent components viz. the vertical axis component (Hz) and horizontal radial component (Hr). The radial component causes the electron traveling in a 2-direction to move in a helical manner concerning the central axis. The axial component causes the electron to move closer to the central axis, i.e. the effect of the axial component is to reduce the diameter of the helical path of the electrons. As a result, the electron beam spirals down the column as it passes through the electromagnetic lens. The resultant effect is that the electron beam becomes finely focused and can be scanned over the sample for imaging purposes.

The scanning of the electron beam over the surface of the sample is achieved by deflecting the beam using an applied electrostatic or magnetic field. Typically, a deflection yoke consists of four radially oriented coils arranged so that the magnetic field is perpendicular to the axis of the system. The magnetic field generated by these coils can be controlled by the amount of electric current passing through these coils. By programming these coils, one can readily focus the electron beam over the sample surface.

The typical accelerating voltage used in SEM is of the order of a few thousand volts. With an energetic beam of electrons scanning over the sample surface, several phenomena occur due to interactions between the electrons and sample atoms. For example; the incident electron can collide with the electron of the atoms or atomic nuclei. Energetic incident electrons can collide with the electrons of the sample and knock them out of their usual orbits. These electrons are known as secondary electrons. During the process, the incident electron loses little energy and continues to generate more secondary electrons as it travels further into the sample. A single incident electron will generate a shower of thousands of secondary electrons until the secondary electron loses its energy. Since a large number of secondary electrons is generated, the detection of the secondary electron is the most common mode of operation for SEM sample imaging. Secondary electrons are having low energy so secondary electrons generated deep in the sample are unable to travel to the surface and leave the sample. As a result, the secondary electrons detected are primarily from a region close to the sample surface (< 10 nm). Thus SEM imaging would produce good topographical information for the sample.

Sometimes, an incident electron collides with the nucleus of a sample atom, causing the electron to bounce back. Such electrons are referred to as backscattered electrons. Since the atomic nucleus is more massive than an electron, the backscattered electrons have high velocity and are characterized by
their high energy of a few KeV. High-density samples will generally create more back scattered electrons, and hence backscattered electrons can be utilized to identify the difference in the densities of the sample. The production of back scattered electrons is directly proportional to the atomic number of the atoms in the sample. Therefore, the regions with atoms of higher atomic numbers would appear brighter than regions with atoms of lower atomic numbers. Thus besides providing the information on topography back scattered electrons provide valuable information on the density and elemental distribution in the sample.

2.9 Transmission Electron Microscope

Transmission Electron Microscope is a type of electron microscope that has three essential components viz. electron gun, image producing system, imaging recording system.

(a) Electron gun: An electron gun is an electrical component that produces an electron beam that has precise kinetic energy. The electron gun at the top of a TEM emits electrons that travel through the microscope or vacuum tube. There are two types of electron gun i.e. thermionic emission and field emission. The primary component of electron gun are the electron emitter, the biasing cylinder and the anode. The tungsten filament is covered by a control grid known as Wehnelt cylinder made up of a central hole which lies columnar to the tube. The cathode lies on top of or below the cylindrical column hole. The cathode and the grid are negatively charged with an end of the anode which is disc shaped that also has an axial hole. When electrons are transmitted from the cathode, they pass through the columnar aperture to the anode at high voltage with constant energy, which is efficient for focusing the specimen to produce an accurately defined image.

(b) Image Producing System: The image producing system is made up of objective lens, a movable stage or holding the specimen, intermediate and projector lenses. They function by focusing the passing electron through the specimen forming a highly magnified image. The objective has a short focal length of about 1-5 mm and it produces an intermediate image from condenser which are transmitted to the projector lenses for magnification. The projector lenses are of two types i.e. intermediate lens which allows great magnification of the image and the second one is projector lens which gives generally greater magnification over the intermediate lens.

(c) Image Recording System: The image recording system is made up of florescent screen used to view and to focus on the image. They have digital camera that can effective record the image and capture after viewing it. They have vacuum system that prevents the bombardment or collision of electrons with air molecules disrupting their movement and ability to focus. A vacuumed system facilitates the straight movement of electrons to the image. The vacuumed system is made up of a pump, gauge, valves and a power supply. The generated image is known as monochromatic image, which is greyish or black in color. The image should be such that it should be easily visible to human eyes. Therefore, it is allowed
to pass through fluorescent screen fixed at the base microscope. The image can be captured digitally and displayed on the computer and stored in a JPEG format.

2.9.1 Working Principle: TEM is a microscopic technique in which a beam of the electron is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. The working principle of TEM is very much similar to the light microscope. The prime difference between light microscope and electron microscope is that, in case of light microscope, light rays are used to focus and produce image whereas in case of TEM, electron beams are used to focus on the specimen and to produce image. Electrons are having shorter wavelength as compared with light. The mechanism of a light microscope is that an increase in resolution power decreases the wavelength of light, but in TEM when electrons are illuminated, the specimen, the resolution power increases increasing the wavelength of the electron transmission. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto the imaging device such as a fluorescent screen. In TEM analysis, a thin specimen is illuminated with electrons in which the electron intensity is uniform over the illuminated area. As the electrons travel through the specimen, they are either scattered by a variety of processes or they may remain unaffected by the specimen. The result is that a non-uniform distribution of electrons emerging from the surface of the specimen contains all the structural and chemical information about the specimen. An electron microscope is constructed to display this non-uniform distribution of electrons in two different ways viz. angular distribution of scattering that can be viewed in the form of scattering patterns, and spatial distribution of scattering that can be observed as contrast in images of the specimen. The advantages of this arrangement are the possibility of directly viewing the area from which the diffraction pattern arises. The basic components of the transmission electron microscope are shown in Fig. 7.
A technical explanation of a typical TEM working is as follows:

a) The vital source i.e. an electron gun produces a stream of monochromatic electrons.

b) TEM works with voltage electron beam. Here a focused beam of electrons instead of light is used to image.

c) The magnification power is over 2 million times better than that of light microscope.

d) TEM uses electron beam for resolution.

e) The stream is focused to a small, thin coherent beam by the use of condenser lenses 1 and 2. The first lens is usually controlled by a spot size knob that largely determines the spot size and the second lens is usually controlled by the intensity or brightness knob that changes the size of the spot on the sample. The second lens usually controlled by the intensity or brightness knob actually changes the size of the spot on the sample.

f) The beam is restricted by the condenser aperture, knocking out high-angle electrons.

g) The beam strikes the specimen and part of it are transmitted.

h) The transmitted portion is focused on the objective lens.

i) Optical objective and selected area metal apertures can restrict the beam, the objective aperture enhancing contrast by blocking out high-angle diffracted electrons, and the selected area aperture enabling the user to examine the periodic diffraction of electrons by the ordered arrangement of atoms in the sample.
j) The photographic image is recorded from the electron after it has passed through a thin sample of
the specimen under study.

k) The image is passed down the column through the intermediate and projector lenses.
The image strikes the phosphorus image screen and light is generated, allowing the user to see the image.
The darker area of the image represents those areas of the sample that fewer electrons were transmitted through.

**Table 10: Distinguish between SEM and TEM**

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SEM creates image by detecting reflected or knocked-off electrons</td>
<td>TEM creates image using transmitted electrons that passes through the sample</td>
</tr>
<tr>
<td>2</td>
<td>SEM provides information about the surface, elemental composition and topography</td>
<td>TEM provides information about inner structure, crystal structure, stress state information of the materials under study using broad beam of electron</td>
</tr>
<tr>
<td>3</td>
<td>SEM has resolution limit to 0.5nm</td>
<td>The recently developed TEM has images has resolution up to 50pm</td>
</tr>
<tr>
<td>4</td>
<td>SEM provides 3D images</td>
<td>TEM provides 2D images</td>
</tr>
<tr>
<td>5</td>
<td>There is no requirement of ultrathin sample preparation</td>
<td>As the electron is transmitted from one end of the material to another end, here ultra-thin sample preparation is essential.</td>
</tr>
<tr>
<td>6</td>
<td>SEM sample requires little or no sample preparation</td>
<td>TEM sample preparation is complex and tedious procedure which can be done by trained skill expert only</td>
</tr>
<tr>
<td>7</td>
<td>SEM operates in the accelerating voltage up to 30Kv</td>
<td>TEM operates in the accelerating voltage up to 60-300Kv</td>
</tr>
<tr>
<td>8</td>
<td>SEM can magnify the sample up to 1-2 million times</td>
<td>TEM can magnify the sample up to 50 million times</td>
</tr>
<tr>
<td>9</td>
<td>Sample is placed at the bottom of the electron column and the scattered electrons are captured by electron detector.</td>
<td>In TEM sample is located in the middle of the column. The transmitted electron pass through it and through a series of lenses below the sample.</td>
</tr>
<tr>
<td>10</td>
<td>In SEM electrons are captured and counted by detectors, image on PC screen</td>
<td>In TEM direct imaging on fluorescent screen or PC screen with CCD</td>
</tr>
<tr>
<td></td>
<td>SEM is faster technique</td>
<td>TEM is slower technique</td>
</tr>
<tr>
<td>---</td>
<td>------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>12</td>
<td>In SEM less sample restriction than TEM</td>
<td>In TEM more sample restriction than SEM</td>
</tr>
<tr>
<td>13</td>
<td>SEM is less versatile than TEM</td>
<td>TEM is more versatile than SEM</td>
</tr>
<tr>
<td>14</td>
<td>SEM is less expensive than TEM</td>
<td>TEM is more expensive than TEM</td>
</tr>
<tr>
<td>15</td>
<td>In SEM surface, powders, polished and etched microstructures, IC chips and chemical segregation are all imaged.</td>
<td>TEM is used to image dislocations, small precipitates, grain boundaries and other defect structures in solids.</td>
</tr>
</tbody>
</table>

2. 10 Combing SEM and TEM Together: There is one more analytical technique which uses combination of both SEM and TEM. This instrument is called as scanning transmission electron microscopy. This instrument can be applied to a TEM tool. Most modern TEM can be switched to STEM mode. In STEM mode, the beam is finally focused and scan the sample area while the image is generated by the transmitted electron.

2.11 Scanning Probe Microscope: A scanning probe microscope is a microscope that produces an image by scanning a solid probe on or extremely close to the surface of a specimen. Some signal is detected from the interaction of the probe with surface, and in principle this signal can be used to form an image. The image is then formed from the setting of the feedback control that is required to keep the signal constant or from a second signal for example the amplitude of oscillation can be controlled by feedback and the phase angle of oscillation used to form an image.

As in SEM, where the probe is focused beam of electrons, the resolution of image is controlled by the region of interaction. The part of the probe which interacts with the sample is very small and in several forms of STM it is small enough to allow atomic resolution. Extremely precise control of position is required in all STMs both in x and y scanning across the surface and for z height control, and this is accomplished by use of piezoelectric drivers. The first STM to be developed was scanning tunneling microscope. In STM the probe is a conductor set at a bias voltage difference from a conducting sample and signal is the current that passes between them. The probe sample interaction is the quantum mechanical tunneling current that has a measurable value only when the two conductors are very small distance apart, typically less than 1nm. In the STM region, the interaction in the specimen can be limited to a single atom, so the microscope can show the atomic arrangement on surfaces. The second type of STM to be developed and currently the most important is atomic force microscopy.
2.12 Atomic Force Microscope: The AFM can be operated in a number of ways. The basic difference between the mode of operation is how much the tip touches the specimen surface, contact mode is when the tip contacts the specimen all the time, intermitted contact mode is when the tip oscillates and touches the specimen some of the time, in non-contact mode the tip does not touch the specimen at all.

2.12.1 Contact and Associated Mode: In contact mode AFM, the tip mounted on the cantilever is in contact with sample surface during scanning. This is similar in principle to profilometer where a stylus is dragged over a surface. In the AFM, a very smaller contact area and highly sensitive cantilever allow high resolution of topographical surface feature, both laterally and vertically. During scanning, the local variation of vertical force for a rigid sample is due to variant ion in the height of the surface. Feedback control of this force causes the vertical position of the tip to trace out the surface topography. Because the tip stays in contact with surface during scanning, the cantilever experiences forces acting not only normal but also lateral to the sample surface. The origin of lateral force is the shear force in the sample at the tip position. The shear force can be very high and can cause damage to the soft polymer or biological samples under study.

The force acting between the tip and sample surface plays a vital role in contact mode AFM, and understanding these force is key to image interpretation in this mode. In feedback operation, the force between tip and sample is kept constant. Controlling the force variation led to development of the force modulation microscope. The up and down movement results in a deflection of cantilever that will depend upon local forces and local mechanical properties of sample.

2.12.2 Intermittent contact mode: The application of contact mode AFM to soft material such as polymers and biological specimen is highly restricted because of their delicate structure which can get damaged due to lateral forces. In IC-AFM the cantilever is forced to oscillate vertically near its resonance frequency, which is typically around 50-499kHz. The amplitude of free cantilever oscillation is typically a few tons of nanometers. The vibrational amplitude is reduced when cantilever is brought closure to the sample surface and begins to touch it. Topographical images are produced as the amplitude depends upon the mean height.

2.13 Electrochemical techniques:

The relationship between current density and over potential is predicted by equation called as the Butler-Volmer equation and plot is called as Tafel plot which determines the deposition potential of metal species present in the electrolyte. If the potential is increased, at the start the current density changes linearly, a point reached at which a small increase in over-potential causes a large increase in the current density. This over potential is called decomposition potential or deposition potential of that species.
When the potential is externally applied across the electrolytic cell, the stability is disturbed and the polarized interface, “double layer” is disturbed, which causes depolarization of the interface. The curves of over potential vs. current density are therefore referred as voltammogram. The voltammogram besides estimating the deposition potential are also useful in understanding the cathodic or anodic reactions and their rates, which ultimately controls the rate of deposition of the particular species. Of all the methods available for studying electrode processes, potential sweep methods are probably most widely used [38].

2.13.1 Cyclic voltammetry

![Cyclic Voltammetry Diagram]

**Fig. 8:** A pictorial representation of the Cyclic Voltmeter.

Cyclic voltametry is an important analytical technique. It is type of potentiodynamic electrochemical measurement. In such type of experiment electrode potential ramped linearly versus time. Here the working electrode potential is ramped in the opposite direction to return to initial potential. It is possible to ramp in potential. The current at the working electrode is plotted versus the applied voltage to give cyclic voltamogram trace. This technique is normally used to study the electrochemical properties of a sample under study in solution condition.

The fundamental behind it is the application of a potential sweep to the working electrode with respect to a reference electrode. In linear sweep voltammetry the potential scan is done only in one direction, stopping at chosen value, $E_t$, for example at $t=t_1$ as shown in Fig 9. In cyclic voltammetry, on reaching $t=t_1$ the sweep direction is inverted and sweep until $E_{\text{min}}$, then inverted and sweep to $E_{\text{max}}$ etc.

The important quantities involved are-Initial Potential ($E_i$), Initial sweep direction, Sweep rate ($v$), Maximum potential ($E_{\text{max}}$), Minimum potential ($E_{\text{min}}$), Final Potential ($E_f$).
Due to the electrode reaction faradic current, \((I_f)\) is noted in the relevant region of applied potential where electrode reaction occurs. There is also a capacitive contribution: on sweeping the potential, the double layer charge changes: This contribution increases with increasing sweep rate \([39]\). The total current is

\[
I = I_c + I_f = C_d \left(\frac{dE}{dt}\right) + I_f = v C_d + I_f
\]  

(10)

Thus \(I_c = v\) and it can be shown that \(I_f = v^2\). This means that at very high sweep rates capacitive current must be subtracted in order to obtain accurate values of the rate constant. The applicability of Nernst equation and therefore, reversibility has to do with time allowed for the electrode to reach equilibrium. The concentration of the species at the interface proportional to the mass transport of these species from bulk solution, frequently described by mass transfer coefficient \(K_d\). A reaction which is reversible corresponds to the case where the kinetics of the electrode reaction is much quicker than the transport. The kinetic is given by standard rate constant, \(K_0\) which is the rate constant when \(E = E^{0'}\)

\begin{align*}
\text{E- Actual potential and } E^{0'} - \text{ Formal potential } [E^{0'} - \text{ standard potential}]
\end{align*}

The kinetics of electrode reactions does not measure the rate of electron transfer itself, as this is an adiabatic process, obeying Frank-Condon principle, and occurs in approx. \(10^{16}\)s. What it measures is the time needed for the species, once they have reached the interfacial region, to assemble themselves and their ionic atmospheres into position for electron transfer to be able to occur. By considering the kinetics of the reaction there are three types: a) Reversible, b) Irreversible, and c) Quasi reversible

\textbf{a) Reversible system}

Fig. 10 shows a characteristic curve for linear sweep voltammetry noted for reversible reaction of the type \(O + ne^- \rightarrow R\). The curve can be understood in the following way. On attaining a potential where the
electrode reaction begins, the current increases as in a steady state voltammogram. However, the formation of a concentration gradient and expenditure of electroactive species means that, continuing to sweep the potential, from a definite value just before the maximum value of the current, peak current, the supply of electroactive species begins to fall. Due to reduction, the current then begins to decay, following a profile proportional to $t^{-1/2}$ similar to application of potential step. Fig. 11 shows the characteristic cyclic voltammetry for reversible system.

![Characteristic curve of linear sweep voltammetry for reversible system](image1)

**Fig. 10: Characteristic curve of linear sweep voltammetry for reversible system**

![Characteristic curve of cyclic voltammetry for reversible system](image2)

**Fig. 11: Characteristic curve of cyclic voltammetry for reversible system**

For linear sweep and cyclic voltammogram of reversible reactions important parameters are-

- $E_p$ independent of $v$
- $E_p - E_{p/2} = 56.6/n$ mV
- $I_p \alpha n^2$ [11]

And for cyclic voltammetry alone

- $E_{pa} - E_{pc} = 59.0/n$ mV
- $|I_{pa}/I_{pc}| = 1$ [12]
In voltammogram the solution resistance between working and reference electrode shifts the potential of the working electrode by \( I_p R \Omega \) (where \( R \Omega \) is the resistance (uncompensated) of the solution).

**b) Irreversible system**

In case of an irreversible reaction of the type \( O + ne^- \rightarrow R \), linear sweep and cyclic voltammetry bring about the same voltammetry profile, since no inverse peak appears on inverting the scan direction.

![Voltammogram for irreversible system](image1)

**Fig. 12 Voltammogram for irreversible system.**

Fig. 12 shows a voltammogram for irreversible system. As compared to reversible system, the waves are shifted to more negative potential (reduction), \( E_p \) depending on the sweep rate. The peaks are broader and lower.

**c) Quasi reversible systems**

The extent of irreversibility rises with rise in sweep rate, while at the same time there is a decrease in the peak current relative to the reversible case and an increasing separation between anodic and cathodic peaks. On increasing sweep rate, there is less time to reach equilibrium at the electrode surface; reactions which appear as reversible at low sweep rates can be quasi reversible at high sweep rates. Fig. 13 shows the effect of increasing irreversibility on the shape of cyclic voltammogram.

![Effect of increasing irreversibility on CV](image2)

**Fig. 13 The effect of increasing irreversibility on the shape of CV.**

**2.14 Electrochemical impedance spectroscopy (EIS)**
It is known to the electrochemistry community for more than a century; Macdonald recently published an excellent account of its history [40]. Electrical equivalent circuits (EECs) describe an electrochemical reaction that takes place at the electrode/electrolyte interface, using an EEC as a model. The current flowing at an electrified interface due to an electrochemical reaction, $Ox + ne^- \leftrightarrow Red$, always contains non-faradaic components, no matter how well the measurement is made. In this equation, $n$ is the number of electrons transferred, $O$ is the oxidant and $R$ is its reduced product (reductant). The electron is transferred across the electrified interface. Fig. 14 shows an electrified interface in which the electrode is negatively charged; counter cations are aligned along the electrified surface. At bottom are the electrical circuit elements corresponding to each interface component. The high-frequency components are shown on the left and the low-frequency components are shown on the right. where, $C_d$–is the double-layer capacitor, $C_E$– is the counter electrode, IHP- is inner Helmholtz plane, OHP- is outer Helmholtz plane, $R_p$- is polarization resistance, $R_s$- is solution resistance, WE-is working electrode, $Z_W$– is Warburg impedance.

**Fig. 14: An EDLC at negative electrode of cell.**

The charge transfer leads to both faradaic and non-faradaic components. The faradaic component arises from the electron transfer via above mentioned reaction across the interface by overcoming suitable activation barrier, namely the polarization resistance ($R_p$), along with the uncompensated solution resistance ($R_s$). Then on faradaic current results from charging the double-layer capacitor ($C_d$). When the charge transfer takes place at the interface, the mass transports of the reactant and product take on roles in determining the rate of electron transfer, which depends on the consumption of the oxidants and the production of the reductant near the electrode surface. The mass transport of the reactants and the
products provides another class of impedance ($Z_w$), which can be oppressed by electroanalytical chemists because it shows up in the form of a peak current in a voltammogram or a current plateau in a polarogram.

Impedance spectroscopy is a powerful method to evaluate a component’s performance in the frequency domain. Special equipment is required to apply a small AC voltage and measure the changes in magnitude and phase over a range of frequencies. An ideal capacitor is represented by a vertical straight line shifted on the real axis by its ESR. At low frequencies a SC approaches a near vertical straight line shifted on the real axis by the ESR.

AC method is commonly used to make a measurement over a wide range of frequencies. The DC value of the conductivity can be extracted from the corresponding AC data. Many AC measurements are performed with blocking electrode such that no discharge or reaction occurs at the electrode-electrolyte interface. Because the current will flow back and forth, no ion pile up is found on the electrode surface, especially when a high AC frequency is used. Commonly used electrodes are platinum, stainless steel, gold and indium tin oxide (ITO) glass.

Impedance is simply the opposition to AC circuit. It contains a real and an imaginary part (i.e. complex). An electrochemical cell shows resistive, capacitive, as well as inductive properties. The resistive property denotes the real part of the impedance, while the capacitive and the inductive properties contribute to the imaginary part of the impedance. In general, an electrochemical cell can be considered to be a network comprised of a resistor, a capacitor, as well as an inductor. The arrangement for such a cell is usually determined after performing a measurement and analyzing the form of the impedance curve. The capacitor presents as an open circuit in a DC network and an inductor that appears as a straight conductor wire in a DC circuit, both appear as imaginary resistors in an AC circuit. The complex impedance can be written in a general form as

$$Z(\omega) = Z'(\omega) - i Z''(\omega)$$  \hspace{1cm} (13)

where, $\omega$- is frequency, $Z'(\omega)$ - is real part of impedance, contributed by the resistive part, $Z''(\omega)$ - is imaginary part of impedance, contributed by the capacitive part, $i=\sqrt{-1}$, the imaginary number.

2.15 Particle size distribution

Particle size distribution is also known as grain size distribution. Particle size of nanomaterials influences many properties. It is a valuable indicator of their quality and possible applications. This concept holds valid for all phases of materials such as powder, suspension, emulsion and aerosol. If the particle size is small, then its solubility increases which leads to higher surface charge (zeta potential). The particle size analysis not only reveals the average particle size but also other statistical distribution parameters such as mode, median, distribution width etc. which are equally important. Particle size
measurement is routinely carried out across a wide range of industries and is often a critical parameter in the manufacture of many products. Particle size has a direct influence on material properties such as reactivity, stability, texture, viscosity, and porosity. Particle size analysis is the statistical distribution of the particles of different sizes. It is common practice to represent this distribution in the form of either a frequency distribution curve or a cumulative distribution curve. Particle size distribution can be represented in many ways such as number-weight, volume-weight and intensity-weight distribution.

2.15.1 **Number-weight distribution:** A counting technique such as image analysis will give a number-weight distribution where each particle is given equal weighting irrespective of its size. This is most important where knowing the absolute number of particle is important.

2.15.2 **Volume-weight distribution:** Static light scattering technique such as laser diffraction will give a volume-weight distribution. Here the contribution of each particle in the distribution relates to the volume of that particle; i.e. relative contribution will be proportional to the \( (\text{size})^3 \). This is very useful from a commercial perspective.

2.15.3 **Intensity-weight distribution:** Dynamic light scattering technique will give an intensity-weight distribution where the contribution of each particle in the distribution relates to the intensity of light scattered by particle; for example, using the Rayleigh approximation the relative contribution for very small particles will be proportional to the \( (\text{size})^3 \).

When comparing particle size data for the same sample measured by different techniques it is important to realize that the type of distribution being measured can produce very different particle size results. This can be illustrated in the example as shown in Fig. 15.

![Fig. 15: Dynamic Light Scattering](image)

2.16 **Surface Area Measurement using BET:**
The surface area becomes important factor at nanoscale. Hence when we consider phenomenon at nanoscale measurement of surface area is an important parameter. At Nano scale there is an increase in
surface area of material. The increased surface area imparts exotic properties to the material at Nanoscale. But why to determine Surface Area? Surface area directly correlates with several factors as under:

a) **Particle Size:** The particle size is a notion introduced for comparing dimensions of solid particles i.e. flakes, liquid particles i.e. droplets, gaseous particles i.e. bubbles. The notion of the particle size applies to the particles in colloids, in ecology, in granular materials and the particles that forms a granular material. The particle size can be measured by various methods such as ultra sound or electric field or gravity or centrifugation method.

b) **Particle:** In nanoscience and technology a particle can be defined as a small localized object which can be described by several physical or chemical properties such as volume, density and mass. Anything that is composed of particle is termed as particulate. But in practice the term particulate is normally used to refer to pollutants.

c) **Morphology:** This is the external appearance of the material. Actually this word is derived from biology. In morphology of nanoparticles, different parameters considered are Surface, Texture, Porosity, and Reactivity.

d) **Catalysis and Separation:** These are the substance which alters the rate of chemical reaction without taking part in it. But the physical state of the catalyst may change at the end of reaction. When the rate of chemical reaction gets accelerated the phenomenon is called positive catalysis. There are some substances which can enhance the efficacy of the catalyst and are known as promoters. In presence of certain substances, the rate of chemical reaction is decreased and they are called as negative catalyst.

2.16.1 **Surface area dependent properties and applications:**

a) **Adsorption:** This is phenomenon of accumulation of one substance on the surface of another substance. On the other hand, absorption can be defined as the phenomenon of penetration of one substance into the bulk or body of another substance.

b) **Pigments:** A pigment can be defined as a powder used to add color or change visual appearance. The pigments are completely or partially insoluble in aqueous medium. The pigments may be organic or inorganic in nature. The organic pigments may e azo pigments which contains Nitrogen groups. These pigments are generally red in color.

c) **Pharmaceutical Products:** The pharmaceutical products consist of active ingredients which are combined with additional materials, selected to control dosage delivery. The pharmaceutical products have been traditionally manufactured in batch facilities permitting the flexible use and operation of equipment for the production of a variety of low volume and high volume products.

d) **Chromatographic carriers:** In case of gas chromatography, choice of mobile phase is important. Hydrogen gas has the range of flow rates that are comparable to helium in efficiency. But helium may
be more efficient and provide the best separation if we optimize the flow rate. Therefore, helium is the most common carrier gas. But, use of helium is not always affordable due to its high cost.

e) Sintered Materials: Sintering or frottage is the process of compacting the forming of a solid mass of material by pressure or heat without melting it to the point of liquefaction. Sintering

2.16.2 Adsorption: It is the phenomenon of accumulation or collection of one substance on the surface of another of another substance. The term adsorption can also be defined as concentration at the interfacial layer between the two phases of a system. The adsorption can be categorized into different types:

a) Positive Adsorption: When there is an increase in concentration of one substance on the surface of another substance, it is said to be positive adsorption.

b) Negative Adsorption: When there is a decrease in concentration of one substance on the surface of another substance, it is said to be negative adsorption.

Difference between adsorption and absorption: There is difference between adsorption and absorption. Adsorption is surface phenomenon while absorption is bulk phenomenon [1].

BET aims to explain the physical adsorption of gas molecules on the solid surface and serves as the basic for the important analytical techniques for the measurements of the specific surface areas of the material [2]. The adsorption can be further sub classified as under:

c) Physical adsorption: Here adsorption of gas molecules takes place due to Physical force of attraction. In physical adsorption formation of multiple layer takes place. The adsorption of molecule takes place due to van der waal’s physical force of attraction.

d) Chemical adsorption: Here adsorption of gas molecules take place due to formation of chemical bonds. In chemical adsorption of monolayer takes place. In chemical adsorption formation of chemical bonds take place. In chemical adsorption, the gas molecules are held on the surface by chemical bonds and a surface compound is formed. Chemical adsorption is categorized by high heat of adsorption \(20-100 \text{ kcal/mole}\) Chemical adsorptions are rarely reversible. To remove the adsorbed a gas the solid must be heated to a higher temperature and pumped at higher vacuum. In chemical adsorption only single molecules of adsorption layer may be formed. It is found only when there is tendency towards compound formation [3]. BET theory explains physical adsorption of gas molecules on the solid surface and serves as the basic technique for the measurement of the specific surface area of the material. Normally nitrogen gas is employed adsorption for surface probing by BET method. Therefore, standard BET analysis is often conducted at low temperature i.e. boiling temperature of \(\text{N}_2\) i.e. 77K.

2.16.3 Theory or Principal -:
The concept of BET is an extension of the Langmuir theory; Langmuir theory is applicable to monolayer adsorption. The postulates are as under:
1) Gas molecules physically adsorption on the solid in layer infinitely
2) Gas molecules only interacts with adjacent layer and
3) Langmuir theory is applicable to each other layer.
4) The desorption is a kinetically limited process i.e. heat of adsorption must be supplied.
5) The uppermost layer molecular layer is in equilibrium with the gas phase.

2.16.4 BET Assumption:
Adsorption takes place on the uniform surface and the energies of adsorption of all molecules in the first layer are identical.
1) Each molecule adsorbed in a layer is itself a potential adsorption site for next layer.
2) There is no steric limitation to the thickness of the multilayer.
3) It is only for the first layer that the differential energy of physical adsorption $E_1$ is higher than the energy of liquefaction $E$.
4) Interaction between molecules adsorbed in the same layer does not play any part in the adsorption equation.
5) All the molecules in the monolayer cover the same area
6) The arrangement of these molecules is assumed to be hexagonal close packed.
7) The molecular cross section area can be derived from density of the adsorption in the bulk liquid state [4].

\[
\frac{P}{v_t(P_0 - P)} = \frac{1}{V_m C} + \frac{C - 1}{V_m C} \frac{P}{P_0}
\]  

(14)

where, $P =$ Equilibrium Pressure
$P_0 =$ Saturation pressure
$V_t =$ Volume of adsorbed gas
$V_m =$ Monolayer of adsorbed gas
$C =$ BET constant

\[
C = e^{\frac{(E_1 - E_2)}{RT}}
\]  

(15)

where, $E_1=$Heat of adsorption of first layer
$E_2=$ Heat of adsorption of second layer

2.16.5 Instrumentation of BET:
The single-point method can be successfully applied for series of samples in powder form. These circumstances may be verified by comparison with specific surface area determined by the single point method. The close similarity between the single point value and multiple point values indicates that $1/C$ approaches to zero.
**a) Sample preparation:** Before the determination of specific surface area of the sample, it is necessary to remove gases and vapors that may have been physically adsorbed on the surface after manufacture and during treatment, handling and storage. This phenomenon is popularly known as outgassing. In case if outgassing is not achieved, the specific surface area may be reduced or may be variable because an intermediate area of the surface is covered with molecules of the previously adsorbed gases or vapors. The outgassing conditions are critical for obtaining the required precision and accuracy of specific surface area measurements on pharmaceuticals because of the sensitivity of the surface of the materials [6].

**b) Conditions:** It is essential to demonstrate outgassing conditions to yield reproducible BET plots. The outgassing conditions defined by the temperature, pressure and time should be chosen so that the original surface of the solid is reproduced as closely as possible. The outgassing of many substances can be achieved by applying a vacuum. Here sample is purged in a flowing stream of non-reactive dry gas. In either case, elevated temperatures are sometimes applied to increase the rate at which the contaminants leave the surface. Caution should be exercised when outgassing powder samples using elevated temperatures to avoid affecting the nature of the surface and the integrity of the sample. If heating is employed, the recommended temperature and time of outgassing are as low as possible to achieve reproducible measurement of specific surface area in an acceptable time. For outgassing sensitive samples, other outgassing methods such as the desorption-adsorption cycling method may be employed.

**c) Volumetric method Principle:** In the volumetric method the recommended adsorbate gas is nitrogen which is admitted into the evacuated space above the previously outgassed powder sample to give a defined equilibrium pressure, $P$, of the gas. The use of a diluent gas, such as helium, is therefore unnecessary, although helium may be employed for other purposes, such as to measure the dead volume. Since only pure adsorbate gas, instead of a gas mixture, is employed, interfering effects of thermal diffusion are avoided in this method.
**Fig. 16: BET Measurement Set-Up**

**d) Procedure:** In the sample tube admit a small amount of dry nitrogen so as to prevent contamination of clean surface. Then remove the sample tube and stoppered is to be removed and weighed. Calculate the weight of the sample. Attach the sample tube to the volumetric apparatus. Cautiously evacuate the sample down to the specified pressure. Alternatively, some instruments operate by evacuating to a defined rate of pressure change and holding for a defined period of time before commencing the next step.

If the principle of operation of the instrument requires the determination of the dead volume in the sample tube, for example, by the admission of a non-adsorbed gas, such as helium, this procedure is carried out at this point, followed by evacuation of the sample. The determination of dead volume may be avoided using difference measurements, that is, by means of reference and sample tubes connected by a differential transducer. The adsorption of nitrogen gas is then measured as described below. Raise a Dewar vessel containing liquid nitrogen at 77.4 K up to a defined point on the sample cell. Admit a sufficient volume of adsorbate gas to give the lowest desired relative pressure. Measure the volume adsorbed, \( V_a \). For multi-point measurements, repeat the measurement of \( V_a \) at successively higher \( P/P_o \) values. When nitrogen is used as the adsorbate gas, \( P/P_o \) values of 0.10, 0.20, and 0.30 are often suitable.

**e) Reference materials:** Periodically verify the functioning of the apparatus using appropriate reference materials of known surface area, such as \( \alpha \)-alumina, which should have a specific surface area similar to that of the sample to be examined.

a) BET method is based on adsorption of gas on a surface.
b) The amount of gas adsorbed at a given pressure allows to determine the surface area.
c) It is cheap, fast and reliable method.
d) It is very well understood and applicable in many fields.
This method is not applicable to all types of isotherms.

### 2.17 Contact Angle Meter (CA)

![Image of a water drop on a Lotus leaf.](image)

**Fig. 17:** Water drop on Lotus leaf.

A definition of contact angle is needed before discussing contact angle measurement techniques. The contact angle can be defined in several ways. Qualitatively, a contact angle is the macroscopic representation of microscopic phenomena. Microscopic characteristics such as surface roughness, surface energies of the materials involved, and surface coatings play a role in the wettability of a material for a given fluid. Quantitatively, a contact angle is the interior angle formed by the substrate being used and the tangent to the drop interface at the apparent intersection of all three interfaces. This intersection is called the contact line. The contact angle is the angle which is conventionally measured through liquid where liquid vapor interface meets. The angle of contact is determined by the interaction across the three interfaces as shown in above diagram. Most often the concept is illustrated with a small liquid droplet resting on a flat horizontal solid surface. The tangent line and angle of contact of a liquid droplet is shown in the diagram. The static angle of contact on a flat surface can be determined by famous Young’s equation. In fact, Young equation is a force balance in the horizontal direction. It is possible to determine ratio of interface surface tension using angle of contact.

#### 2.17.1 Typical contact angles:

Let us see the possible way of interaction of a liquid droplet with solid surface. There may be strong attraction of liquid droplet with solid substrate surface or there may be weak attraction of liquid droplet with the solid substrate surface. When a liquid is very strongly attracted to the solid substrate surface the droplet will completely spread on the solid substrate surface and angle of contact will be acute and close to 0. On the hand if the droplet of liquid is having less affinity with the solid substrate surface it will not penetrate inside. The liquid droplet will not spread. It will remain in the form of droplet only for longer time. In such cases the angle of contact is higher than (0. Sometimes angle of contact may be as high as 150-180. In such case the liquid will not wet the surface. Such materials are called as a
hydrophobic material if liquid is water. These materials are super hydrophobic materials. The super hydrophobic materials can be obtained on fluorinated surfaces like Teflon’s. This phenomenon is popularly called as Lotus effect. The contact angle thus directly provides information on the interaction energy between the surface and the liquid. Sometimes the contact angle is measured through the gas instead of through the liquid, which reverses 0 and 180 in the above explanation.

2.17.2 TECHNIQUES FOR MEASURING CONTACT ANGLES
There are several methods of determining contact angles. The shape of the droplet is determined by the Young Relation. The contact angle plays the role of a boundary condition. Contact angle is measured using a contact angle goniometer. The contact angle is not limited to a liquid/vapor interface; it is equally applicable to the interface of two liquids or two vapors. Companies such as Kyowa have developed equipment to measure microscopic contact angle and dynamic contact angle.

2.17.3 Different Measuring methods for determination of contact angle:
* Static Sessile drop method
* Dynamic Sessile drop method
* Powder contact angle method
* Single fiber Wilhelmy method
* Dynamic Wilhelmy method

Different methods are having different requirements of the aggregate sample according to the different measurement theories. Regarding the measurement of surface free energy of aggregates different methods are uses as: Wilhelmy method, wick method also known as capillary rise method, micro-calorimeter method, universal sorption device, and inverse gas chromatography.

2.17.4 Factors affecting angle of contact: The angle of contact depends upon various factors as below:
* Surface roughness
* Porosity
* Impurities
* Functional groups present on the surface
* Surface energy

a) The static sessile drop method:
The sessile drop method is commonly used for determination of angle of contact. Here the angle is measured with contact angle goniometer. Higher value of contact angle indicates low solid surface energy and chemical affinity. Whereas low contact angle indicates high solid surface energy and higher chemical affinity which results in complete wet ability. Here the liquid used for experimentation is called as probe liquid.
b) **The dynamic sessile drop method:**

The dynamic sessile drop is very much similar to the static sessile drop method. Here actually the drop is modified where as in case of static sessile method regular droplet is used. The contact angle is affected by surface chemistry and surface roughness. When surface is rough the droplet is in wenzel state i.e. it creates homogeneous wetting whereas cassie-baxter state i.e. it creates heterogeneous wetting. This maximum angle is the advancing angle. Volume is removed to produce the smallest possible angle, the receding angle. The difference between the advancing and receding angle is the contact angle hysteresis. These methods are very straightforward in nature.

c) **Dynamic Wilhelmy method:**

A method for calculating average advancing and receding contact angles on solids of uniform geometry. Both sides of the solid must have the same properties. Wetting force on the solid is measured as the solid is immersed in or withdrawn from a liquid of known surface tension.

d) **Single-fiber Wilhelmy method:**

Dynamic Wilhelmy method applied to single fibers to measure advancing and receding contact angles.

e) **Powder contact angle method:**

Enables measurement of average contact angle and sorption speed for powders and other porous materials. Change of weight as a function of time is measured.

2.18 **Photoluminescence Spectroscopy:** This is the contactless, non-destructive method of probing the electronic structure of material. Here light is incident on the sample, where absorbed excess energy is
imported into the sample. This phenomenon is called as photo-excitation. The excess of energy is imparted on the sample and can be used for different ways.

- Increase in internal energy of the sample.
- Excess amount of energy is reflected.
- The incident energy is transmitted to neighboring molecules.
- The phenomenon of luminescence is observed.

2.18.1 Luminescence: It is a process of spontaneous emission of light due to some cause other than heat. Luminescence may take place as a result of chemical reactions, electrical energy, subatomic motion or stress on the crystal. On another hand incandescence is the phenomenon of emission of electromagnetic radiations from a hot body. Thus incandescence is the thermal emission. As in case of luminescence, spontaneous emission of electromagnetic radiations takes place without heating. Thus, sometimes radioactivity is also considered as luminescence. There are various types of luminescence such as bio-luminescence, chemi-luminescence and another type is called photoluminescence. The phenomenon of emission of light can take place as a result of biochemical reactions taking place inside the living organisms. The example includes production of light from glow worm. There are number of marine animals which emits light as a result of biochemical reactions such as star fish, jelly fish etc. Similarly, as a result of chemical reactions sometimes light is generated such type of phenomenon is known as chemi-luminescence.

When electron at ground state absorbs energy it gets excited to higher energy state. High energy state is unstable. Electron resides in higher energy state for short span of time and then returns to ground state by emission of energy. Depending upon amount of energy absorbed electron can be promoted to various higher energy states. While coming back to lower energy state excess energy can be released in the form of heat and light.

\[ S_n \rightarrow S_1 \] It is called internal conversion and energy is released in the form of heat.

\[ S_n \rightarrow T_1 \] It is called as inter system crossing and this phenomenon is iso-energetic. Hence energy is not given out.

\[ S_1 \rightarrow S_0 \] This phenomenon is called as fluorescence. Here energy is emitted in the form of light.

\[ T_n \rightarrow S_0 \] This phenomenon is called as phosphorescence. Here energy is released in the form of light.

The phenomenon of fluorescence and phosphorescence can be well explained with the help of Jablonski diagram as below:

3 Nanotechnology in Agriculture and Veterinary Science:
Being aware with the beneficial aspects of Nano-medicine in the domain of veterinary sciences and animal husbandry, veterinarians and scientists are actively trying to harness the application of materials
at nanoscale in the diagnosis, prevention and treatment of diseases. The aim of this research paper is to gather the widespread applications of Nano medicine and to highlight standard research practice in the veterinary medicine field. In recent years’ nanotechnology showed good results in diagnosis, disease treatment, gene therapy, regenerative medicine, oncology, dentistry, drug delivery and therapeutics. For over centuries, silver-based compounds were used as non-toxic inorganic antibacterial agents owing to their biocidal properties in many applications such as wood preservatives, water purification in hospitals, in wound or bur healing. Silver ions and their related compounds have low toxicity towards animal cells but exhibit very high toxicity towards microorganisms like bacteria and fungi. Also, silver-based compounds are much cheaper than gold-based compounds. In recent years, the antimicrobial properties of Ag nanomaterials gradually aroused people's concern and a lot of antimicrobial applications were reported. The antimicrobial properties of silver nanoparticles with different sizes were studied by Agnihotri et al. From the experimentation it is observed that silver nanoparticles with 5 nm show the best antimicrobial efficacies. It has been observed that the smaller particles exhibit better antimicrobial effects. It is also observed that silver in combination with some antibiotics increases the efficacy of the antibiotics. Nanoparticles are already used in numerous applications including in vitro diagnosis. But the use of nanoparticles in medicine is the prime focus of research.

3.1 Biomedical Applications of Nanotechnology

- **Bio-sensors:** A bio-sensor is the device that measures biological or chemical reactions by generating signals proportional to the concentration of analytes in the reaction. The bio-sensors are employed in applications such as disease monitoring, drug discovery and detection of pollutants and markers that are indicators of a disease in body fluids such as urine, blood, saliva and sweat.

- **Genetic alteration:** Nanoparticles can also be used in conjugation with DNA to perform genetic alteration. Nanoparticles are significantly less toxic than currently used organic methods, providing more efficient method of monitoring genetic alteration.

- **Cell Interaction:** Cellular interactions occur at microscopic level and this cannot be observed even with the help of advanced microscopes. The development of new branch nanobiotechnology has opened the doors to study the interactions at molecular level.

- **Nano-medicine:** This is the sub-discipline of veterinary medicine that is likely to contribute significantly and imminently to overcome the challenges and obstructions in veterinary medicine.

- **Nano-robotics:** It is an emerging technology that creates machines or robots whose components are at molecular scale. The nano-medicine could use these nano-robots introduced into the body
to repair or detect the damaged or infected parts. Carbon could be the primary material required to design the nano robots.

- **Tissue Engineering**: Tissue engineering is an interdisciplinary science which is a branch of biotechnology that involves the use of biological sciences and engineering to develop tissue that restore, maintain or enhance tissue function. The term tissue was coined by anatomist and pathologist Xavier Bichat in 1801, whereas the term tissue engineering was coined by Granlibakken in 1987. The aim of tissue engineering is to control pathologies through artificially facilitated tissue regenerative processes. At present tissue engineering plays very small role in treatment. The regenerative medicines are hope for tomorrow for incurable diseases. Eugene Bell is considered as father of tissue engineering. With the advancement in the sector bladder, small arteries, skin graft, cartilage and trachea have been developed and implanted successfully in the patients. The materials development in tissue engineering includes use of three dimensional bio-printing, organ on chip, and induced pluripotent stem cell technologies. The application of nanomaterial is the fast growing field of tissue engineering.

- Nanomaterials in veterinary pharmaceutical sciences: Nanotechnology has highly affected pharmaceutical sectors. The efficacy of pharmaceutical drugs can be significantly improved with the help of materials at nanoscale. The materials at nanoscale have complex pharmacokinetics and dynamic behavior in vivo, requiring a careful consideration of their dose regime. The researchers are always interested to minimize the possible adverse effect of the drug and to minimize the concentration and frequency of the medicinal drugs.

- Nanoparticles bound drugs to have an extended half lifetime in vivo, longer circulation times and can convey a high concentration of potent drug to the location where it is needed

- Medical nano-devices could be injected in the human body and would then go work in a specific organ or tissue mass. The doctor will monitor the progress and make certain that nano devices are at correct position. Nano-robots pay a crucial role for many applications in the human body such as a targeting tumoral cells for therapeutic cells.

- The nanomaterials can be used in bioremediation as they can degrade the pollutants without affecting eco-system, since nanomaterials are synthesized from natural products.

- Nanoparticles are valuable tools in identification of biomolecules through the use of bio-tagging and labelling. Attachment of ligands or ligands or molecules coating to the surface of a nanoparticle facilitate NP- molecule interactions and make them bio-compatible.

- **Medical devices**: Neuro-electronic interfacing is the construction of nano-devices which can permit computers to be joined and linked to the nervous system. This concept has led the building of a molecular structure that will permit control and detection of nervous impulses by
an external computer. A nanoscale enzymatic biofuel cell for self-powered nano-devices have been developed that uses glucose from bio-fluids including human blood.

- **Nano-hybrid**: Materials developed at nanoscale has progressed from single particle synthesis to multi-component synthesis. Nano-hybrid is a term reserved for material where two or more pre-synthesized nano-materials are conjugated to design a multifunctional material. The nano-hybrids are materials with organic and inorganic component that are linked together by non-covalent bonds or covalent bonds at nano-meter scale. The nano-hybrid materials can be classified into following four categories viz (a) carbon-carbon nano-hybrid, (b) carbon-metal nano-hybrid, (c) metal-metal nano-hybrid, (d) organic molecules coated nano-hybrid.

- **Drug formulation**: The term drug is derived from French word drogue meaning dry herbs. A drug is an agent which is intended for the use in diagnosis, mitigation, treatment, cure or prevention of diseases. In pharmaceuticals, the drug formulation is the process in which different chemical substances, including the active drug are combined to form a medical product. The drug may vary from their route of administration eg external formulation: oral drugs are normally taken as tablets, capsules, suspensions or power. The drug itself need to be soluble in aqueous solution at a controlled rate. The dissolution is affected by various factors such as particle size, crystal formation, etc. The rate of dissolution may be fast or slow depending upon condition. Here it is worth to mention that fast dissolution is not always favorable. The slow dissolution rate can prolong the duration of action or avoid the high initial plasma level. Treatment of active ingredients by special way such as spherical crystallization can have some advantage for drug formation. The drug is stored in liquid or in unstable lyophilized form.

- **Parenteral formulation**: Some of the drugs are absorbed poorly when consumed orally. Such types of drugs must be administered in some less desirable ways like intravenous injection or by inhalation. These are termed as injectable formulations and are used with intravenous, subcutaneous, intramuscular and intra-articular administration. The drug is stored in liquid or in unstable lyophilized form. Many parenteral formulations are unstable at elevated temperature and hence they are stored at refrigerated or sometimes in frozen conditions. The example of topical formulation includes such as (a) crams: These are emulsion of oil and water in approximately equal proportion and is capable of penetrating the stratum, outer layer of the skin etc., (b) ointment: These are combination of oil and water. Here oil and water unequally mixed. Here proportion of oil is about 80% and that of water is about 20%, (c) gel: These type of medicine liquefies when comes in contact with skin, (d) paste: Such type of medicine is having three type of phase viz oil, water and power. In another words we can say these are ointments in which power is suspended, (e) power: These are finely subdivided solid drug.
Dendrimers: These are highly branched symmetrical, monodispersed thread like structure with well-defined number of end groups which can be functionalized to exhibit unique applications. The dendrimers encapsulated metallic nanoparticles are stable and hence they do not agglomerate. The branched structure of dendrimers are also allowed for small molecules to enter their core to get access to the metallic nanoparticles. The dendrimers can work as drug carriers in animal cells. The dendrimers shows potential applications in human and veterinary medicine e.g. diagnosis, artificial enzymes, sensors and molecular weight standards, light harvesting, inks and tenners, organo-silicane coating, nano-composite, pharmaceutical and bio-medical applications.

Biomedical Imaging: In vivo imaging is very important domain of research and development in veterinary science and animal husbandry. With the advancement in science and technology various tools and devices are being developed. The nanoparticle contrast agents can be used to develop the images. In cardiovascular imaging, nanoparticles have potential to aid visualization of blood pooling, ischemia, angiogenesis, atherosclerosis and focal area where inflammation is present. The small size of nanoparticles has the properties that can be very useful in oncology especially in brain tumors. When quantum dots are used in conjugation with MRI we are getting exceptional images of tumor sites. CdSe nanomaterials glow when they are exposed to ultra-violet radiations. When these nanomaterials are injected they seep into cancer tumors. The quantum dots can produce higher contrast image at lower cost than today’s organic dye stuff. However, it is important to note that quantum dots are usually made up of very toxic materials. The dot can be random in size and made up of bio-inert material and they can demonstrate the nanoscale properties that color is size dependent.

Protein cage: This is the naturally derived cage that is stable in physiological conditions and is bio-compatible as a drug carrier. One of the known example of protein cage is ferritin and apoferritin. When iron atom is removed from ferritin apoferritin is formed. An important property of ferritin/ apoferritin is that its 24 subunits can dissociate at pH 2.00 of the solution and reconstruct into an intact shell structure as the pH of the solution is slowly adjusted to neutral.

Formation of Artificial tooth: A real bone is the actually nano-composite material, composed of hydroxyapatite, crystallites in the organic matrix, which is mainly composed of collagens. The bone mechanically touches and at the same plastic. The bones can get repaired from mechanical injuries. The actual nano-scale mechanism leading to this useful combination of properties still not properly understood. An artificial hybrid material was developed from 15-
18nm ceramic nanomaterials and poly methyl methacrylate co-polymers. The visco-elastic behavior of human teeth was demonstrated with the help of tribology.

- **Nano-motors:** A nano-motor is the molecular or nanoscale device capable of converting energy into movement. Natural biological motors are present in the cytoskeleton, the DNA or RNA processing enzymes or bacterial rotatory fragments. There are three types of motor protein assembly (1) dynein, (2) kinesine and (3) cilia.

- **The materials at nanoscale are important materials in the development of novel nano-devices which can be used successfully in numerous physical, biological, biomedical and pharmaceutical applications.** The size of the drug nanoparticles and their surface characteristics can be modified to achieve the desired delivery characteristics.

- **Nanoparticles bound drugs cannot be effectively circulated, thus high localized circulation can be achieved.**

- **As the properties of materials at nanoscale are different from that of bulk naturally efforts have been made to capture some of those properties by incorporating nanoparticles into composites for instance modern rubber tire which is typically composite of a rubber and an inorganic filler such as carbon black or silica nanoparticles.**

- **Blood purification:** The purification of blood is based on functionalized iron oxide or carbon coated metal nanoparticles with ferromagnetic or superparamagnetic properties. The binding agents are many times organic molecules such as proteins, antibiotics, antibiotics or synthetic ligands are covalently linked to the particle surface. The binding agents can interact with target molecules and forms the agglomerate. When an external force is applied the gradient exerts a force on the nanoparticle. Therefore, the particles can be separated from the bulk fluid, thereby cleaning it from the contaminants.

- **DNA Nanotechnology and drug delivery systems:** For the appropriate delivery of the veterinary drugs DNA based drug delivery devices have been introduced in recent years such as DNA guns and DNA vaccines. In order to increase cellular intake efficacy, efficient drug biomolecules such as doxorubicin and CpG oligonucleotides, have been successfully amalgamated with DNA based nanostructure to increase cellular intake efficacy.

- **The bed sore is the common condition in bed ridden persons.** When a person is paralyzed or suffering from incurable or terminal diseases and cannot walk or stand then it is possible bed sore can be developed. It is difficult to treat bed sore as one portion of the body experiences continuous high pressure. There are number of creams like Neosporine or Arnica available in the market for the treatment of wound. But many times these wounds are severe and does not get healed. It is possible that nanomaterials can be effectively used in wound healing. The nano-
crystalline silver dressing, gels, and creams effectively reduce bacterial infections in a chronic wound. The silver nanoparticles containing poly-vinyl nano-fibers also show the efficient antibacterial property in wound dressing.

- Nano-biotechnology and Gene therapy: For the treatment of different type of diseases new approach has been followed such as nano-biotechnology and gene therapy. Here DNA molecules are introduced into patient cell to replace defective or missing gens. In this method it is possible to treat genetic disorders and other diseases. The development in the gene editing technology uses nanoscale tools to modify precisely DNA sequence and make corrections in genetic mutation.
- Silver sulfadiazine depicts better healing of burn wounds due to its slow and steady reaction with serum and other body fluids.
- In the surgical wards there is always possibility of infection. Therefore, it is very much essential to maintain aseptic conditions in the operation theatre and in admission wards. Attempts are made to keep the operation theatre, ward and devices in aseptic condition. This could be achieved by various means such as sterilization, antiseptic lotions like Dettol etc. However, with the advancement in the nanoscience and technology the researchers developed many materials which could be directly used to reduce the risk of infection. Silver impregnated medical devices like surgical masks, apron, and implanted devices show significant anti-microbial activities.
- Anti-inflammatory Agents: Inflammation is the part of body’s defense mechanism. This is the process by which body recognizes and removes harmful stimuli and starts the healing process. Based upon visual observations the inflammation shows five distinct signs viz. redness (rubor), swelling (tumor), heat (color), pain (dolor), loss of function (funcio laesa). Inflammation is not a symptom of infection. As far as treatment possibility is concerned rest, ice and good wound care is recommended. In the traditional way of treatment Ascorbic acid, Calciferol and zinc is generally given. The natural remedies for inflammation is turmeric, ginger and green tea. In certain autoimmune disease, such as rheumatoid arthritis, which is a chronic syndrome characterized by rapid progression, swelling, inflammation and ultimately destructs the joints. The available medication for treatment are found to be initially beneficial but after some days the conventional treatment shows many side effects. Thus, it is advisable to administer the drugs directly at the site of inflammation. But this is an expensive process. This can be achieved using nanoparticles as the vector which can target and administer the anti-inflammatory drugs directly on the site of swelling. This is seen in many cases where NPs as encapsulated drugs containing liposomes are used to deliver drug such as glucocorticoids and clodronate, targeting arthritis in animal models.
Nanotechnology against parasitic diseases: There are various affecting the livestock due to micro-organisms. These parasitic diseases are due to protozoa, fungus, bacteria or virus. After the discovery of antibiotics veterinary doctors used antibiotics for the treatment of diseases. The first antibiotic Penicillin was discovered British Biologist Sir Alexander Fleming and he was awarded Nobel prize for this wonderful life saving discovery. But later on it was realized that the effectiveness of antibiotics gets decreased after frequent uses. Also it is recommended to complete the full cycles of antibiotics. However, after frequent uses the pathogenic micro-organisms develop a resistance towards the particular antibiotics and becomes ineffective to control the diseases. This becomes a great challenge in front of veterinary doctors to treat the animals. Many efforts are being made to overcome this problem. In this context several new organic compounds are discovered and shows potential activity for a particular period in order to control the disease. The nanomaterials show a new hope for control of diseases due to microbes. The genomic and proteomic fields are already contributing much to the elucidation of molecular insight into disease, and with the assistance of nanotechnology, new opportunities are being put in the hand of veterinary doctors to develop better and improved diagnostic tools with the power of genetic elucidation of irregularities at the level of genes.

- Silver zeolite can be used in food preservation, disinfection, and decontamination of products.
- Nanoparticles that exhibit antimicrobial activity had also been incorporated into paints and coatings, making those products particularly useful for surfaces in hospitals and other medical facilities and in areas of food preparation.
- Forty years of revolutionary interaction among biology, medicine and nanotechnology have led to present day nano-biotechnology, which is now showing progressive application in multiple aspects of the medical field. From disease detection to treatment, many veterinary medicine issues such as disease diagnosis, drug discovery, personalized medical procedures, are now improving on the use of nano-biotechnology.
- Silver nanoparticles have been commonly found to have broad-spectrum activities against pathogens.
- Various nanotechnology based diagnostic kits such as nano-sensors, nanoparticle based PCR Assay, Lab on chip devices, along with modern drug and medicine such as nanoparticles base drug delivery vehicles are being commercialized for clinical uses and also for research.
- Silver nanoparticles are larvicides against filariasis and malaria vectors.
- Silver nanoparticles have been shown to prevent viruses such as HIV binding to host cells.
• Silver sulfadiazine is a combination of silver and sulfadiazine. Silver sulfadiazine is used as a broad-spectrum antibiotic. All kinds of sulfa drugs have been tested in combination with silver. But sulfadiazine was found to be most effective.

• Nanotechnology---- Antiviral and Antibacterial Applications: These diseases arise due to pathogenic virus or bacteria. The virus or bacteria affect at microscopic scale. Thus, it is very effective to combat with such diseases with materials at nanoscale. If we look back into history, we find ancient Indian philosophers and doctors were aware with the medicinal use of materials at nanoscale. Today an upgraded version of nanoscale based material conversion is shown to improve the efficacy of such traditional and modern medicinal options.

• Nanotechnology in Regenerative Medicine and Stem Cells: Regenerative medicine deals with the process of replacing, engineering or regenerating human or animal cells, tissues or organs to restore or establish normal function. This is an interdisciplinary branch of science. The field of regenerative medicine encompasses numerous strategies, including the use of materials and de novo generated cells, as well as various combinations thereof, to take the place of missing tissue, effectively replacing it both structurally and functionally or to contribute to tissue healing. Materials are often an important component of current regenerative medicine strategies because the material can mimic the native extracellular matrix of tissue and direct cell behavior, contribute to the structure and function of new tissue, and locally present growth factor for example decellularized donor tissues are also used to promote wound healing. Incorporation of growth factors that promotes healing or regeneration into biomaterials can provide the local and sustained presentation of these factors, and these approach has been exploited to promote wound healing by delivery of platelets derived growth factors and bone formation via delivery of bone morphogenic proteins 2 and 7.

• Administration of cell can induce therapeutic responses by indirect means, such as secretion of growth factors and interactions with the host cells or having transplanted cells from a bulk tissue. For example, infusion of human umbilical cord blood cells can aid in stroke recovery due to enhanced angiogenesis.

• The transplanted macrophages can promote liver recovery. The transplanted cells can also normalize the injured or diseased environment and improve tissue regeneration. Here cells are often infused, typically intravenously, in current clinical trials, but cells administrated in this manner often experience rapid clearance, which shows its limited efficacy.

• Infused cells can be modified genetically to express a targeting ligand to control their biodistribution.
In the area of health and veterinary sciences magnetic nanoparticles are extensively used to replace radioactive technetium for tracking the spread of cancer along lymph nodes. The nanoparticles work by exploiting the change in contrast brought about tiny particles of super-paramagnetic iron–oxide in magnetic resonance imaging.

Nanoparticles can be designed to enhance fluorescent imaging or to enhance images from positron emission topography or ultrasound.

The bio-synthesized methods are advantageous because sometimes the nanoparticles are coated with lipid layer that confers physiological solubility and also stability.

The development of nanoparticles to aid in the delivery of a drug to the brain via inhalation holds considerable promise for the treatment of neurological disease like Parkinson’s disease, Multiple sclerosis or Alzheimer’s disease.

As far as tissue or organ implantation is considered, nanoparticles and nanofibers play an important role in the design and development of novel scaffold structure tissue and bone repair. For instance nanomaterials of calcium hydroxypatite, a natural component of bone, is used in combination with collagen or collagen substitutes.

It is possible that such nanoparticles can successfully kill tumors via hyperthermia, in which an alternating magnetic field cause them to heat and destroy tissue on local scale.

3.2 Application of Nano-particles in Agriculture: Green synthesized nanomaterials have number of potential applications in agriculture so as to increase the productivity of agriculture of products. Today the farmers of developing countries are extensively using chemicals such as herbicides, pesticides, fertilizers etc. to control the pest and enhance the yield of economical crops. In fact many of these pesticides, herbicides and chemical fertilizers are carcinogenic in nature. Researcher N R Prasad lost his beloved father recently due to brain tumor. It is suspected that consumption of resin or some other vegetable source might have affected and could have resulted in development of tumor. If pesticides, herbicides or chemical fertilizers in nano forms are used then there may be lower risk of accumulation of hazardous chemicals inside the body of an individual. The nanomaterials are commonly used for generating products such as nano-pesticides, nano-fungicides, nano-biosensors, nano-fertilizers. The nano-biosensors could be developed for agriculture sectors. The nano-biosensors includes enzymatic biosensors, geno-sensors, apta sensors and immuno-sensors which are constructed using wide range of electro-chemical, biological or physico-chemical transducers. The nano form of pesticides with metallic nanomaterials reveals stronger effect against phyto-pathogens, insects and other pests that threaten crops. Today fungus is the most brutal pest for plants and it causes more than 70% of crop damage. To control the fungus, farmers are freely using fungicides. The long term exposure of these fungicides leads to environmental pollution and dangerous effects on environmental systems. A fertilizer can be defined...
as natural or synthetic substance that contains chemicals necessary to improve plant growth and productivity and improve the natural fertility by overcoming micronutrient deficiencies. But when there is tremendous exposure of fertilizers it damages soil quality which ultimately affects agriculture production. Therefore, there is real need to develop any option which can stop damage of soil. And nano-fertilizers are new hope for researchers and farmers. The nano-fertilizers provide gradual and controlled release of nutrients to target sites through direct internalization of products, which prevent nutrients from interacting with soil, water, air and micro-organisms. The use of pro-biotic microorganisms to generate nanoparticles is an eco-friendly as well as economical process. Lactobacillus and Bifidobacterium are most popular probiotics present in dairy products. Such organisms can be used in production of wide range of products. The earlier research reveals that negative surface electro-kinetic potential of Lactobacilli causes rapid absorption of cations, which in turn is responsible for bio-synthesis of materials at nanoscale. The tendency of Lactobacillus to grow even in presence of oxygen makes them highly viable. It is possible to change the condition in desired directions by adjusting the redox potential in culture medium. Thus changing the composition and reaction condition of culture medium plays significant role in bio-synthesis of metallic nanomaterials. The metallic nanomaterials such as silver, cadmium, gold, selenium, iron, zinc etc. have potential applications in agricultural sciences. Gold nanoparticles are widely used in agriculture as antifungal and anti-bacterial agents and as drug delivery vehicles of fertilizers and pesticide sensors. Copper is an essential micro-nutrient which is present as co-factor in many enzymes and have substantial role in plant metabolism and plant nutrition. Copper nanomaterials are showing anti-fungal activity against various plant pathogen fungus. Selenium is very much essential for proper function and healthy life of livestock, human and plants. Selenium nanoparticles improves plants ability to inhibit pathogens and activates antifungal properties. Standardization of selenium in soil is very important and to achieve this pure selenium compounds are used as fertilizers.

3.3 Industrial Application of Nano-particles:

- Silver nanoparticles and their complexes can be used for solar cells to enhance photoelectrical conversion efficiency and photovoltaic performances.
- The presence of silver nanoparticles increased unique Plasmon resonance optical scattering properties.
- Flame Retardation: Nanoparticles were explored for their potential to replace additives based on flammable organic halogens and phosphorus in plastics and textiles.
- Application of biogenic silver nanoparticles in wastewater treatment: An emerging technology for the abiotic degradation of recalcitrant compounds involves zerovalent metal or bimetallic nanoparticles. Zero valent metals have been used for reductive removal or transformation of
organic compounds. In the area of water purification, nanotechnology offers the possibility of efficient removal of pollutants and germs. Today nanoparticles, Nano-membranes are used for the detection and removal of chemicals and biological substances

- Batteries and super-capacitors: These are the energy storage devices. Batteries can be defined as the ability of engineered nanocomposite material to have very high internal surface area for the storage of electrical charges in the form of small ions or electrons. The nano-composite materials have been synthesized for various applications for the formation of electrodes.

- MEMS: This is an abbreviation. The long form of MEMS is micro-electro-mechanical system. This is the technology of microscopic devices particularly those with moving parts. This merges with nano-scale as Nano-electro-mechanical systems. The MEMS are made up of components between 1 to 100 µ. The MEMS generally consists of central processing unit. There are several components that interacts with the surrounding such as micro-sensors. The fabrication of MEMS evolved from the process technology in semi-conductor device fabrication i.e. the basic technique. The MEMS do not require reagents or a fluidity based substrate to react upon. The MEMS are mainly used in drug delivery systems. The MEMS can be used to visualize the problems in gastro-intestinal tracts.

- Nanocomposites are employed in the development and design of new material serving for an instance building blocks for new dielectric and magnetic materials.

- Nano-clays are incorporated into polymers to improve their strength and impact resistance.

- Water is essential inorganic component for the perpetuation of life on the planet. However, unfortunately most of the natural reservoir of water becomes polluted. The water pollution creates many water borne diseases. Further in hazardous toxic heavy metals dissolved in water destroys the internal organs of the body. There are various possible methods to remove the dissolved heavy inorganic materials from water sample. The Fe₂O₃ attached Ag nanoparticles can be used for the water purification and later they can be removed using a magnetic field to avoid contamination of water.

- Silver nanoparticles can be used for water filtrations.

- Inorganic composites of nanoparticles can be used as a preservative in various products.

- Applications of AgNPs on catalysis: When silver nanoparticles are added into the reaction system the speed of chemical reaction gets altered. Thus, the nanocatalyst of silver nanoparticles has been a rapidly growing research area that involves the use of nanoparticles as a catalyst. We are pretty aware that metals like Ag, Au, Pt, and other metal ions can catalyze the decomposition of H₂O₂ to oxygen.
• Silver nanoparticles have many other applications in various fields such as nanoscale detection and photovoltaic cells.

3.4 Recent Advances in Silver Nanoparticles: Colloidal silver is the colloidal solution of silver nanoparticles suspended in a liquid. This colloidal solution is widely used by physicians. Colloidal silver is marketed as alternative medicine. Colloidal silver products remain available in many countries as dietary supplements. Also in homeopathic medicine Argentinium silver is used in different potencies. Silver nanoparticles can be incorporated in wound dressings. It has been observed that silver catheters and endotracheal breathing tubes may reduce the incidence of catheter-related urinary tract infections and ventilator-related pneumonia. The silver ion is bioactive and in sufficient concentration kills bacteria in vitro. Silver and silver nanoparticles are used as antimicrobial in a variety of industrial, healthcare, and domestic applications.

3.5 Nanomaterials in Energy Storage Devices: Energy is the capacity to do work. There are various forms of energy. According to first law of thermodynamics, 'Energy can neither be created nor be destroyed but one form of energy can be converted into another form of energy'. The growth of human civilization requires continuous expenditure of energy. Herein we are going to discuss particularly electrical energy. In modern period electrical energy is the enormous and flexible form of energy. The electrical energy can be obtained from various sources such as coal, wind, solar energy, tidal energy and chemical energy etc. The researchers are always trying to develop efficient energy storage devices. Ragon plot indicates the different types of energy storage devices such as capacitors, batteries and super-capacitors. Each of the energy storage device has its own advantages. But today for the storage of electrical energy super-capacitors are widely used. There are various types of super-capacitors. One of the type of super-capacitor is based upon metal oxides. With the advancement in the nanoscience and technology various transition metal or transition metal oxide based materials are used in the manufacturing of super-capacitors. Herein we would like to mention certain transition metal and metal oxides in the construction of super-capacitors.

Table. 11 Transition Metal Oxide Electrodes for Energy Storage Devices - Super-capacitors

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Material</th>
<th>Morphology</th>
<th>Csp (in Fg-1)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CuO</td>
<td></td>
<td></td>
<td>[107]</td>
</tr>
<tr>
<td>2</td>
<td>CuO</td>
<td>micro-woolen</td>
<td>346</td>
<td>[108]</td>
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<tr>
<td>3</td>
<td>CuO</td>
<td>Rod-like morphology</td>
<td>300</td>
<td>[109]</td>
</tr>
<tr>
<td>4</td>
<td>hybrid CuO–PAA</td>
<td>Island like structure</td>
<td>136</td>
<td>[110]</td>
</tr>
<tr>
<td></td>
<td>Material</td>
<td>Structure/Formation</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-----------------------------------</td>
<td>--------------------------------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>CuO</td>
<td>well-adherent and porous cauliflower structure</td>
<td>[111]</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>CuO</td>
<td>Nano-buds</td>
<td>[112]</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>CuO/Cu(OH)2 hybrid</td>
<td>Nano-flower (diameters-4-5μm)</td>
<td>[113]</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>CuO in [HPMIM][Cl] IL</td>
<td>Nano-buds with bunch of nano-petals-like structure</td>
<td>[114]</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>CuO/Cu(OH)2 hybrid</td>
<td>Lotus like morphology</td>
<td>[115]</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Ru doped CuO</td>
<td>Nano-crystal structure</td>
<td>[116]</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>CuO</td>
<td>Hierarchical multilayer nano-sheets</td>
<td>[117]</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Hybrid Cu-PAA</td>
<td>Circle like structures</td>
<td>[118]</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>CuO-PAA/CNT films</td>
<td>Circle like structures</td>
<td>[119]</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>CuO</td>
<td>Nano-sheets</td>
<td>[120]</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>GN/macroCuO</td>
<td>macro-structured porous</td>
<td>[121]</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>CuO-GO</td>
<td>globular shaped nanoparticles</td>
<td>[122]</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>CuO</td>
<td>Nano-sheets arrays as well as flower like cluster</td>
<td>[123]</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>NSA–Ag/CuO</td>
<td>Nano-sheets</td>
<td>[124]</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>CuO</td>
<td>nanowires and nano-sheets and flower-like structure</td>
<td>[125]</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>CuS</td>
<td>Nanowire</td>
<td>[126]</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Cu2O/CuO/RGO</td>
<td>Nanoporous particles</td>
<td>[127]</td>
<td></td>
</tr>
</tbody>
</table>
Environmental Aspects and Toxicity of Nanomaterials:

As we have discussed in above sections, today nanoscience and technology become the integral part of our life. Nanoscience and technology is used in from food science to electronics and in nano-medicine. The nanoparticle emission in the environment can take place anytime. Though there are several advantages, and of course due to advantages only, tremendous research in nanoscience and technology are globally going on. But we should realize possible threats of nanomaterials and their applications. Herein we are trying enlist some possible hazardous and toxic effects of nanomaterials particularly in health. The toxicity mechanism depends upon the nature of nanoparticles. But most the nanoparticles show toxicity by the mechanism of oxidative stress. Performing toxic-kinetic and bio-distribution studies in animal models are essential to correlate the potential effects of nanoparticles on human being. The cytotoxic, genotoxic, tumorigenesis, reproductive toxicity and neurotoxicity of nanomaterials are widely studied by various research groups. The effect of transition metal Cu, Ag and Au nanoparticles have been studied on earth worm. The effect of these nanoparticles have been studied on growth, mortality, reproduction rate, genetic changes, tissue damage etc. The several studies based on biological models and biomarkers reveals the toxic effects of nanomaterials particularly ZnO, SiO2, Silver, Titanium dioxide, carbon nanotubes etc. The research study reveals there is cell death, production of oxidative stress DNA damage, apoptosis and inflammation is observed. The nanoparticles can be accumulated in the female genital organ and affect the reproductive capacity of female. Due to the nature of nanoparticles, the main target organs to be affected as liver, kidney and lungs. A wide number of study reveals potential toxicity in the living being which can affect cell structure by the exposure and intake of nanomaterials.

Table 12: Possible Risk of Nanomaterials:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Nanomaterials</th>
<th>Possible Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbon nanomaterials, Silica nanoparticles</td>
<td>Pulmonary inflammation, Granulomas and fibrosis</td>
</tr>
<tr>
<td>2</td>
<td>Silver and Gold Nanomaterials</td>
<td>Distribution into other organs including central nervous system</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>Quantum dots, Carbon and TiO$_2$ Nanoparticles</td>
<td>Skin penetration</td>
</tr>
<tr>
<td>4</td>
<td>MnO$_2$, TiO$_2$ and carbon nanoparticles</td>
<td>May enter into central nervous system through nasal epithelium olfactory neurons</td>
</tr>
<tr>
<td>5</td>
<td>TiO$_2$,Al$_2$O$_3$,Carbon black, Co and Ni nanoparticles</td>
<td>May be more toxic than the micron size particles</td>
</tr>
</tbody>
</table>

**Environmentally Benign Effects of Nanomaterials:**

The materials at nanoscale can be effectively used so as to reduce soil pollution like remediation process to diminish the employment of pollutant products. The nanomaterials can be used in fertilizers, plant nutrients, herbicides, pesticides and growth promoting factors. As we are using the materials at nanoscale, its efficacy increases and also less amount of material will be required. This creates a positive impact on the environment.

**4 Future Scope in Nanoscience and Technology:**

- **Self-healing structure:** If crack appears on bulk materials, changing their structures at nanoscale can give them amazing novel properties viz. water repellent, properties. It is possible to disperse nanomaterials throughout the bulk structure so as to fill the cracks. Thus, it retards the possibility of cracks from tuning huge one.
- **Though nanomaterials are small, they can be efficiently used in various electronic devices. The materials at nanoscale can be used in modern flat television or advanced laptop.**
- **Cosmetics:** There is ever increasing scope of nanomaterials in modern cosmetics. In UV protection already materials at nanoscale are used.
- **Nanomaterials use high precision water filters, corrosion resistant metal substitute etc.**
- **In modern world, there are various sources of information. We are regularly getting data from various sources such as newspapers, journal, survey, electronic devices such as television and radio broad casting and also from WhatsApp. We need such a technology which can process and find patterns in it to solve real world problems efficiently. The nanomaterials can be engineered**
in such a way to create ultra-dense memory to store data in less time without compromising the reliability.

- Some types of nanomaterials can be effectively used to tackle environmental problems. Materials such as carbon nanotubes can produce electricity in an eco-friendly manner and hence are more efficient.

5 Conclusion

In the recent past there has been rapid change in the characterization techniques for biological and nanomaterials. Also the existing technique has advanced due to adaptation of new technology. Today large number of researcher are working in the research and development of nanomaterials. Nanomaterials has entered in veterinary medicine, health and agriculture. As nanomaterials are beyond the perception of human eyes, it is very much essential to have proper characterization technique for the analysis of materials at nanoscale. Thus, keeping this in mind we have tried to focus on the recent development in some of important characterization techniques required in nanotechnology, biotechnology, veterinary sciences, health and pharmaceutical sciences. In the last section we have discussed various applications of materials at nanoscale especially their uses in veterinary science and agriculture. Overall the manuscript would be highly beneficial for the researchers, students and industrial persons working in nanoscience and technology.

6 Conflict of Interest

There are no conflicts of interest to declare.

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