CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter includes a well gathered literature review of studies in the fields of bio-medical nanocomposites, nanotechnology and nanoparticles, production of nanoparticles, and analytical methods for nanoparticles. Details of the information and conclusions from earlier investigations that are relevant to this study undertaking are provided.

2.2 BIO-MEDICAL TEXTILE

Bio-medical textile is a sub-discipline of bio-medical engineering and as per name focuses on the development of textile products and structures for biological and medical applications. Infection caused by bacteria leads to pathogen cross-infection and smell production when the textile material is worn adjacent to the skin. Microbial assault is also responsible for the staining and loss of performance qualities of textile substrates. Health-care professionals' uniforms are thereby given a crucial consideration as they get quickly polluted. Hence, the undesirable microorganisms for human health can proliferate in the textile structures especially fabrics worn next to the skin, due to their intrinsic qualities. Chemical mechanisms, in addition to substrate architecture promote microbial development. Humidity and heat continue to worsen the situation [9].

Medical textile goods are in high demand in both developed and developing nations. With the increased threat of new strains of germs and viruses, such bio-medical items have been proved to be quite effective in first aid, clinical, and sanitary settings. Invariably textile materials play a vital role in the development of acceptable structures for the healthcare and medical sectors. The usage of medical textile goods has increased dramatically, not only in the hospital and healthcare sectors, but also in the provinces utters hygiene is essential; hotels, residences, clubs, gems and other public places [10].

Bio-medical textiles are divided into four categories based on their application areas: a) protective and health-care textiles, b) External hygiene products, c) Implantable materials, and d) Extracorporeal devices [1]. The bifurcations of commonly used items in these groups are illustrated in Figure 2.1.

Bio-medical textiles are made out of a basic textile material with bioactive qualities added to it. The kind (e.g. cotton, viscose rayon, lycra, etc.) and form (e.g. fibre, yarn, fabric) of base textile material vary depending on the appropriateness in a certain end use region, and

many times more than one material variation discovered acceptability for a particular application area. Table 2.1 summarises some of the marketed application areas as well as the basic material used for the purpose.

The important factors to be considered while designing bio-medical textile composites are as follows:

Function: The textile material must fulfil the specific purpose/function for which it was intended, e.g. Swabs require adequate absorbency.

Biocompatibility: *R*esponse of the textile material while making contact with blood and tissue in the body is a criterion considered for this measure. Extent of the reaction varies depending on whether the touch was made inside the body or externally. Internal implanted devices are subject to more stringent rules than exterior implantable devices because they have a higher potential for reactivity [11]. For instance, an artificial ligament is permanent and reacts with blood cells and surrounding tissue, but an external bandage is just temporary and only contacts the exterior skin tissue.

Cost: This variable is influenced by one or a combination of cost influencing participating elements such as raw materials, manufacturing method, and so on. But it should always be specified by the product's end-use performance criteria. Just for an example: cost for producing exterior and disposable surgeons' gowns and swabs should be invariably kept cheaper. Conversely internal articles like vascular grafts, artificial skin etc. is quite expensive.

Product approval: Before bringing medical devices, instruments, and accessories etc. into use, one should get medical and legal permission. For medical textiles, each nation has its own established set of rules and regulations.

Thus, the bio-medical product regardless of its application area; internal or external use, should fulfil precisely above-mentioned criteria, defined based on the individual end-use. They should, however, always include fundamental bioactive capabilities, particularly antibacterial properties [1].

Table 2.1: Base Textile material used in marketed application area [1]

A. Non-Implantable Materials	B. Implantable Materials	C. Healthcare/hygiene Products
Absorbent pad • Non-woven made up from Cotton, viscose, or Lyocell	**Sutures** • Monofilament, or braided Biodegradable made up from Collagen, catgut, polyglycolide and polylactide fibre	**Surgical gowns** • Woven, or nonwoven made up from Cotton, polyester fibre, polypropylene fibre
Wound-contact layer • Woven, non-woven, or knitted made up from Alginate fibre, chitosan, silk, viscose, lyocell, or cotton	**Non-Biodegradable Sutures** • Monofilament, or Braided biodegradable made up from Polyester fibre, polyamide fibre, PTFE fibre, polypropylene fibre, polyethylene fibre	**Surgical caps** • Nonwoven made up from Viscose
Simple non-elastic and elastic bandages • Woven, or non-woven made up from Cotton, viscose, Lyocell, Polyamide fibre, or elastomeric-fiber yarns	**Artificial tendon** • Woven, or braided made up from PTFE fibre, polyester fibre, silk, collagen, polyethylene fibre, polyamide fibre	**Surgical masks** • Nonwoven made up from Viscose, polyester fibre, glass fibre
High-support bandages • Woven, non-woven, or knitted made up from Cotton, viscose, Lyocell, or elastomeric fiber Yarns	**Artificial ligament** • Braided made up from Polyester, carbon fibre, collagen	**Surgical drapes, cloths** • Woven, or nonwoven made up from Polyester and polyethylene
Compression Bandages • Woven, non-woven, or knitted made up from Cotton, viscose, Lyocell, or elastomeric fiber Yarns	**Artificial skin** • Nonwoven made up from Low density polyethylene fibre Artificial cartilage Chitin	**Surgical hosiery** • Knitted made up from Cotton, polyester fibre, polyamide and elastomeric fibre yarns
Orthopaedic Bandages • Woven, non-woven, or knitted made up from Cotton, viscose, Lyocell, polyester, polypropylene, or polyurethane foam	**Eye-contact lenses and Artificial cornea** • Poly (methyl methacrylate) fibre, silicon fibre, collagen	**Blankets** • Woven, or Knitted made up from Cotton, polyester fibre
Plasters • Woven, non-woven, or knitted made up from Cotton, viscose, plastics film, polyester fibre, glass fibre, polypropylene fibre	**Artificial joints/ bones** • Silicone, polyacetal fibre, polyethylene fibre	**Sheets, pillow cases** • Woven made up from Cotton
Gauze dressing • Woven, non-woven, or knitted made up from Cotton, viscose, lyocell, Alginate fibre, Chitosan	**Vascular grafts** • Woven, or knitted made up from PTFE fibre, polyester fibre	**Uniforms** • Woven made up from Cotton, polyester fibre
Wadding • Non-woven made up from Viscose, cotton linters, wood pulp	**Heart valves** • Woven, or knitted made up from Polyester fibre	**Protective clothing, Incontinence diaper /sheet, Cover stock** • Nonwoven made up from Polyester fibre, polypropylene fibre
Scaffold • Spun laid, or needle punched Nonwoven made up from Polylactide fibre, polyglycolide fibre, carbon		

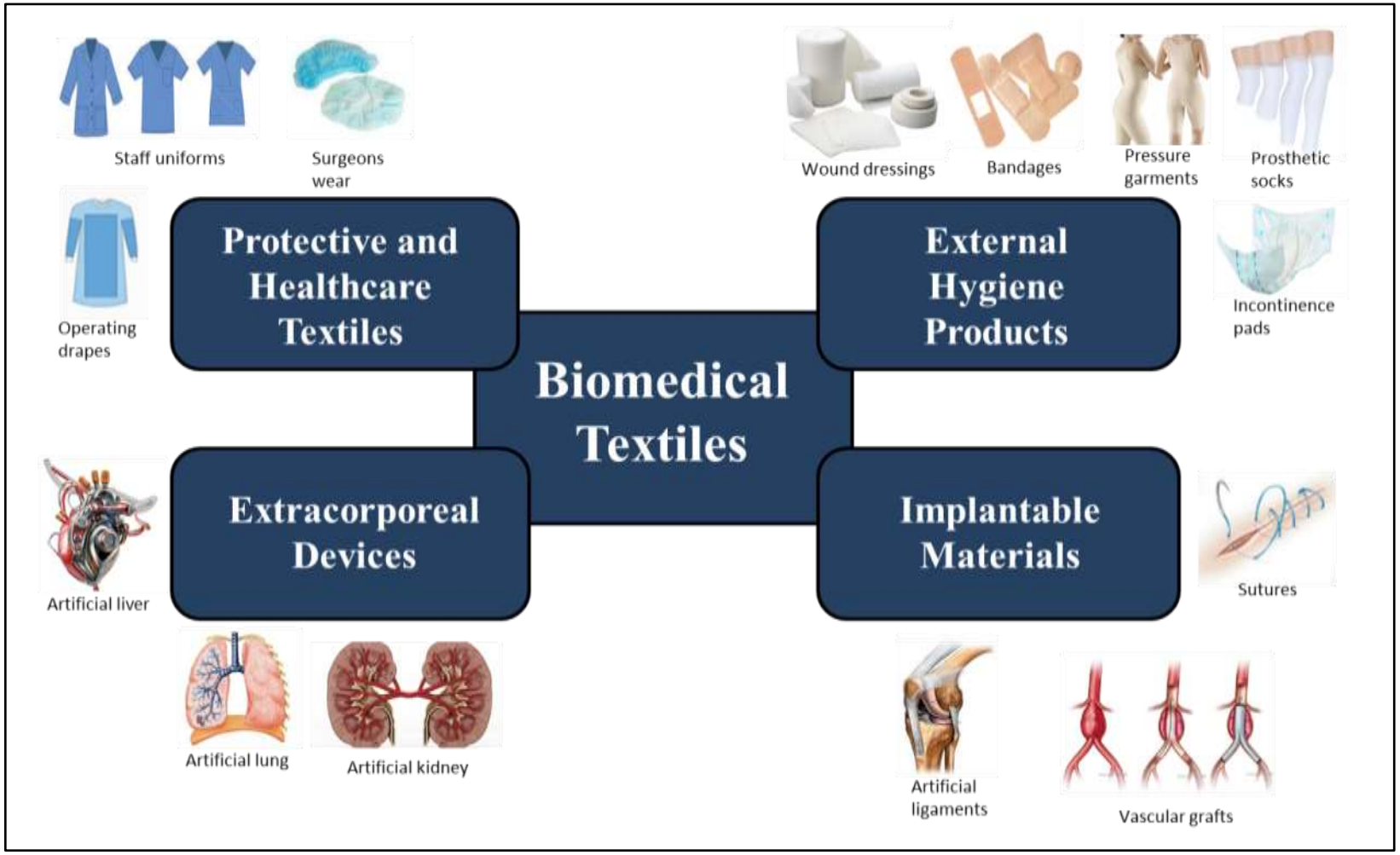


Figure 2.1: Application areas of bio-medical textiles [1]

2.2.1. BACTERIA/MICROORGANISMS IN BIO-MEDICAL TEXTILES

Bacteria, a kind of microorganism, are microscopic organisms that are too small to be seen with the naked eye yet may have a significant influence on human health. Bacteria require resources such as food or water to thrive. Water is collected from humid air, human sweat, other bodily fluids, or a wet cloth, whereas food is retrieved from skin cells and dust. Bacteria are single-celled or unicellular organisms and found mainly in three shapes: spherical (cocci), rod-shaped (bacilli), and spiral-shaped (spirillum), and can be found in pairs, clusters, or chains. Bacteria have a hard layer and a cytoplasmic membrane [12].

2.2.1.1 Type of bacteria

Bacteria are classified as gram-positive or gram-negative based on the composition and structure of their cell walls, as determined by a staining method known as gram-staining [12]. The bacteria are gram-positive if they remain purple after the process; if they turn pink, they are gram-negative [13].

Gram-positive bacteria: Peptidoglycan and teichoic acids are found in Gram-positive bacteria. Peptidoglycan, which is made up of amino acids and sugar, makes up 90% of the cell wall [12]. Teichoic acids are responsible for the organism's antigenic determinant. S. aureus, which occurs in pairs, short chains, or grape-like clusters, is an example of Gram-positive bacteria. It has a size range of 0.5 μm to 1.0 μm. The ideal temperature for growth is between 35 and 40 °C. S. aureus is the most common source of cross-infection in hospitals, accounting for 19% of all surgical infections [14]. S. aureus can also cause boils, skin infections, pneumonia, and meningitis, particularly in the elderly or disabled [15]. Scaled skin and toxic shock syndromes are also caused by it.

Gram-negative bacteria: Gram-negative bacteria are identical to Gram-positive bacteria, with the exception that lipoproteins bind an extra layer of outer membranes to their peptidoglycan layer [12]. Lipopolysaccharide and porin make up the outer layer. Porin is a protein that transports molecules with a low molecular weight. Klebsiella pneumoniae is an example of Gram-negative bacteria (K. pneumoniae). It has the form of a bacillus and comes in single, pair, or short chains. In persons with weakened immune systems, K. pneumoniae is the most common cause of urinary tract infection. The germs can be transported to the lungs by the fecaloral pathway, mouth,

throat, and breath [13]. Fever, trouble breathing, chest discomfort, and bloody stool are all signs of K. pneumoniae.

Escherichia coli (E. coli) is the another example of Gram-negative bacteria. Human intestines are home to E. coli, which is shaped like a bacillus. It can be spread by touching and eating uncooked foods. E. coli causes severe diarrhoea, especially in youngsters, as well as renal damage. Gram-negative bacteria have a thicker cell wall than Gram-positive bacteria, making them more difficult to eradicate. Murray and colleagues found that, Gram-negative bacteria (such as E. coli and Pseudomonas aeruginosa) are more difficult to eradicate than Gram-positive bacteria (i.e., S. aureus) [16].

2.2.1.2 Spreading of bacteria: Bacterium cannot travel from one site to another without the help of a carrier, such as blood, sweat, alcohol, lost skin, or dust. Liquid or air might be used to carry the carrier. Liquid transmission allows germs to be transferred wet or moist. Air transportation is a type of dry transportation that takes place in the air, commonly through vents [14]. Bacteria may be transported via the air by dust, particulates, and lost skin. Humans are known to shed 1,000 germs every minute on an average, which may be a substantial source of contamination in a medical setting [17].

2.2.2. PREREQUISITE FOR ANTIBACTERIAL BIO-MEDICAL TEXTILES COMPOSITES

Medical and allied sector as well as healthcare and hygiene product manufacturing industries are raising stars for the textile industry due to their constantly increasing as well as substantial share. A hospital has an enormous variant for textiles products, as well as the threat of increased traffic. Patients and medical personnel both are always at high point danger of disease transmission and other health associated difficulties because of constant traffic of individuals, particularly those with infectious diseases. The growing prevalence of drug-resistant bacteria emphasises on development of safe and long-lasting antibacterial materials [11].

The growing need for comfortable, aesthetically pleasing, long-lasting, useful, and safe textile goods necessitates promoting the development of innovative and cutting-edge textile manufacturing and designing techniques. Microbes such as bacteria and fungus developed more speedily on the healthcare personnel' (e.g. doctors, nurses, patient, visitors

and others) clothing as they easily get soiled and contributes significantly to the spread of illnesses. Furthermore, germs have been proven to thrive and persist on materials routinely used in hospital contexts for more than ninety days, adding to disease transmission [18].

Antibacterial is a broad phrase that refers to any substance that kills or controls germs. Antibacterial treatment is a process that prevents bacteria from growing in textile fabrics. Antibacterial treated materials are useful not just in medical settings, but also in everyday situations [19]. Bacterial/microbial contamination of surfaces, particularly textile materials, is a common concern in hospitals and healthcare facilities, which can lead profoundly towards infection and cross infections. These occurrences, intimately demands use of antibacterial protective apparel and hospital linens [20].

Textile antibacterial treatment has become more and more significant in the manufacturing of protective, technical, and ornamental fabrics/composites. This has allowed growth of textile applications in the fields of medical, pharmaceutical, engineering, food, and agricultural industries also in parallel. The antibacterial fabric/composite has gained substantial traction in recent years owing to their widespread adoption as surgical wear, infant clothes, and undergarments witnessed this grooming. Hence, the germs can breed easily on textiles that come into close touch with human flesh; such as inner clothes, has the potential to harm both the users and the textile itself [21]. Under such circumstances use of pure textile material will not serve the purpose, it should be a composite textile produced in combination with active membrane group which infuse antibacterial and infection resistant characteristics into them as per the need of application area.

2.2.3. METHODS FOR ANTIBACTERIAL FINISHING/TREATMENTS

A range of health care applications has sparked interest in antibacterial coatings employing fabrics with better functionality. Antibacterial coatings made from natural sources day by day more popular as they encourage a natural and eco-friendly lifestyle. Many infectious illnesses have been recognised to be treated with herbal medicines throughout the ancient days' history. Natural items can be chosen for biological screening based on therapeutic usage of plants. In many underdeveloped nations, they yet continued to play an important role in basic early care as therapeutic medicines [22].

Pad and dry curing, exhaust, spray and foam processes, and coating all technologies are found efficient enough to load functional antibacterial compounds to the textile surfaces.

The ingredients can also be mixed into the fibre spinning dope directly. The commercial agents, it is said, may be used online during the dyeing and finishing processes. There are a number of ways to improve the finish's durability, including:

i. In-solubilisation of the active chemicals in or on the fibre.
ii. Using resin, cross-linking agents, or condensates to treat the fibre.
iii. Antimicrobial agents are microencapsulated in the fibre matrix.
iv. Coating on the surface of the fibre.
v. Chemical modification in the fibre by covalent bond formation.
vi. Graft polymers, homo-polymers, and co-polymerization are also used on the fibre

Dip and Dry Method: This finishing practice has been expanded to textiles usages including outdoor, healthcare, sports, and leisure fabrics. In comparison to synthetic agents, herbal products appear to have intermediate effectiveness with no or low toxicity and are less costly. The cloth is dipped into a bath containing herbal extract for prerequisite period at room temperature before being dried at room temperature in the dip and drying process [23].

Exhaust Method: Treating/finishing textile materials by using the exhaust process prior to dyeing the material might be mordant. The plant-extracted solution was injected to the treated material. Exhaust way injection is usually applied in the jigger drum and other places. Application of the functional matter via pad and exhaustion is said to be permanent thereby proving more effective antibacterial finishing technique than the other examined [24].

Microencapsulation: Microencapsulation is a type of micro packaging in which an active core material is encased in a permeability-limited polymer shell. It's a procedure in which minute particles or droplets are encased in a coating to generate miniature capsules with a variety of beneficial features. On a micro metre scale, it is also integrated in food components, enzymes, cells, and other industrial based items [25]. Furthermore, it is the creation of a barrier to prevent chemical reactions and allow the contents to be released in a regulated manner.

The word microencapsulation is the most suitable since the particles are exceedingly tiny; microcapsules or microspheres are particle sizes between 3 and 800 mm [26]. Microcapsules are made by depositing a thin coating of polymers over small solid liquid particles, or by dispersing solid particles in liquid. Friction, pressure, diffusion through the polymer walls, or diffusion of the polymer covering can all cause the core components in the shell to be freed [27].

Nanoencapsulation: The art and science of manipulating matter at the nanoscale to create innovative and distinctive materials and products is known as nanotechnology [28]. It is also described as the utilisation of structures with at least one Nanometer dimension for the manufacturing of electronics, materials, or systems with novel or considerably enhanced characteristics as a result of their Nano-size. It provides excellent opportunities in textiles, material science, electronics, mechanical engineering, optics, energy, medicine, and aerospace, among other sectors [29].

Coating nanoparticles on the surface of fibres or textiles, according to Yadav et al [30], is one of the strategies for creating high active surfaces with specific features as well as a high durability function for fabrics. Nanotechnology extends new and improved methods for imbuing materials with a variety of useful properties. In fact, textile industrial sector is one amongst the top industries excelled fantastically with the development of wide range products enriched by nanotechnology-based functional characteristics [31].

Wrinkle resistance, water repellency, antibacterial effect, soil resistance, antistatic, UV-protection, flame retardation, improvement of dye ability, electrical conductivity, photo catalytic ability, photo oxidising capability against biological and chemical species, UV absorption, self-decontamination, and blocking functions for both military and civilian heals are some familiar illustrations of the properties infused to the textiles via nanotechnology. In textile finishing, nano metal oxides such as Ag, Au, Al_2O_3, TiO_2, ZnO, SiO_2, MgO, and ceramics are utilised to change the surface characteristics and impart functional qualities [32].

2.3 NANOTECHNOLOGY AND NANOPARTICLES

The term 'nano' is derived from the Greek word 'nano's which means 'dwarf'. However, in the context of nanotechnology, the word 'nano' denotes a billionth (1×10^{-9}), hence the phrase 'nanometre' refers to a billionth of a metre [33]. Nanotechnology is concerned with various matter structured with dimensions of a billionth of a metre or less [34]. The interest in nanotechnology (low-dimensional system technology) is a manifestation of Feynman's famous phrase, "There's Plenty of Room at the Bottom". Based on Feynman's ideas, K. E. Drexler proposed the concept of "molecular nanotechnology" in his book Engines of Creation in 1986, where he proposed utilising tiny molecular structures to work in a machine-like manner to direct and trigger the synthesis of bigger molecules [34].

There are numerous definitions put forward for nanotechnology and its products, which are developed in due course of time for specific reasons. Nanotechnology refers the science and technology used for creating nanoparticles. Simple word it deals with manufacturing machines for the particles having sizes within the range of nanometers.

Nanoparticles represent any microscopical particle less-than about 100 nanometers (nm) in diameter. According to the UK Royal Society and Royal Academy of Engineering Committee, the 'Nanoparticle' ranges from the atomic level, at around 0.2 nm, up to around 100nm, which used for the scope of Nanoscience and nanotechnology [35]. Material possesses significantly different properties in this size range compared to its larger sizes. This happens mainly due to steeper rise in surface area to mass ratio and active quantum effects at these dimensions, leading to significant changes in the physical properties of the material [36].

There are various terms used in association with nanotechnology apart from nanoparticles, their definitions as per publicly available Specification of British Standards Institution's (BSI) Vocabulary are as follows [37];

Nanoscale: with at least one dimension in the order of 100 nm or less.

Nanotechnology: the use of scientific knowledge to alter and control matter at the nanoscale in order to exploit size and structure dependent features and phenomena not found in individual atoms or molecules or bulk materials.

Nanoparticle: particle with one or more dimensions at the nanoscale.

Nanomaterial: a material with one or more exterior dimensions or an interior structure that may exhibit unique properties when compared to the identical material without nanoscale features.

Nano-ecomposite: a composite in which at least one of the phases has at least one nanoscale dimension.

Nanostructure: possessing a nanoscale structure.

2.3.1 FORTITUDE OF NANOTECHNOLOGY

There are many examples of structures with nanometre dimensions (i.e. nanoscale) in the natural world, including essential molecules within the human body and food components, and even though many technologies have inadvertently involved nanoscale structures for many years, it has only become conceivable in the last quarter-century to actively and intentionally change molecules and structures in this size range. However, the characteristics of nanoparticles differ substantially from those of bulk particles. As a result, nanotechnology distinguishes itself from other areas of technology by its capacity to govern at the nanometre scale.

The advancement of nanotechnology has transformed every element of science, engineering, and technology. Nanoparticles' multifaceted properties have made them viable materials for applications in a wide range of fields in recent years. The fields of science and engineering in which phenomena occurring at nanometre scales are used in the design, characterisation, manufacture, and application of materials, structures, devices, and systems.

Many of these applications comprised new materials executing dramatically different qualities while used at the nanoscale. Here word “new” relates with phenomena of the extremely huge surface area to volume ratios encountered at nano dimensions, as well as quantum effects, not realized at the larger sizes. Figure 2.2 demonstrate the remarkable commercial impact observed on the evolution of the nanotechnology. However, one should always remember that their manufacturing incorporates use of harsh, poisonous, and costlier chemicals [39].

Since nano products are enhancing functionality to an unbelievable extent at negligible cost, different types of nanotechnology have shown a greater qualitative and quantitative impact on the society. The list of daily use commercial items with enhanced performance induced via nano-scale materials or nano-technologies is vast. Such widespread products of current commercial market are summarized below;

Nanoscale additives or surface treatments: Fabric surface treatments given on nanoscale can offer lightweight ballistic energy deflection in personal body armour, as well as resistance to wrinkling, staining, and bacterial development [40].

Nanoscale coating: Clear nanoscale coatings applied on eyeglasses, computer and camera displays, windows, and other surfaces can add to their functionalities, like; making them water- and residue-repellent, antireflective, self-cleaning, UV or infrared light resistant, antifog, antimicrobial, scratch-resistant, or electrically conductive [41].

Nanoscale materials: Introducing nanoscale constituents in the structure can enable washability for long-lasting "smart fabrics" outfitted with flexible nanoscale sensors and electronics capable of health monitoring, solar energy collection, and energy harvesting through movement, which were not possible otherwise [42].

Nano-engineered materials: Superior functionality empowered household products became possible with nano-engineered materials, just few popular examples; degreasers and stain removers, environmental sensors, air purifiers, and filters, antibacterial cleansers, and specialty paints and sealing solutions such as self-cleaning house paints that resist dirt and stains.

These revolutionary rewards of the nanotechnology are credited to its amazing capacity to bring silent characteristics of macro form materials on the surface when used in the nano form. Just for an example making material further stronger, lighter, durable, reactive, sieve-like, or better electrical conductors on nano treatment [43].

The sectors most readily embracing nanotechnology (Figure 2.3) are Information and communications sector, electronic and optoelectronic fields, food technology, energy technology, and the medical products sector; which includes many different facets of pharmaceuticals and drug delivery systems, diagnostics. In general, use of nanotechnology will be extremely advantageous to both individuals and organisations.

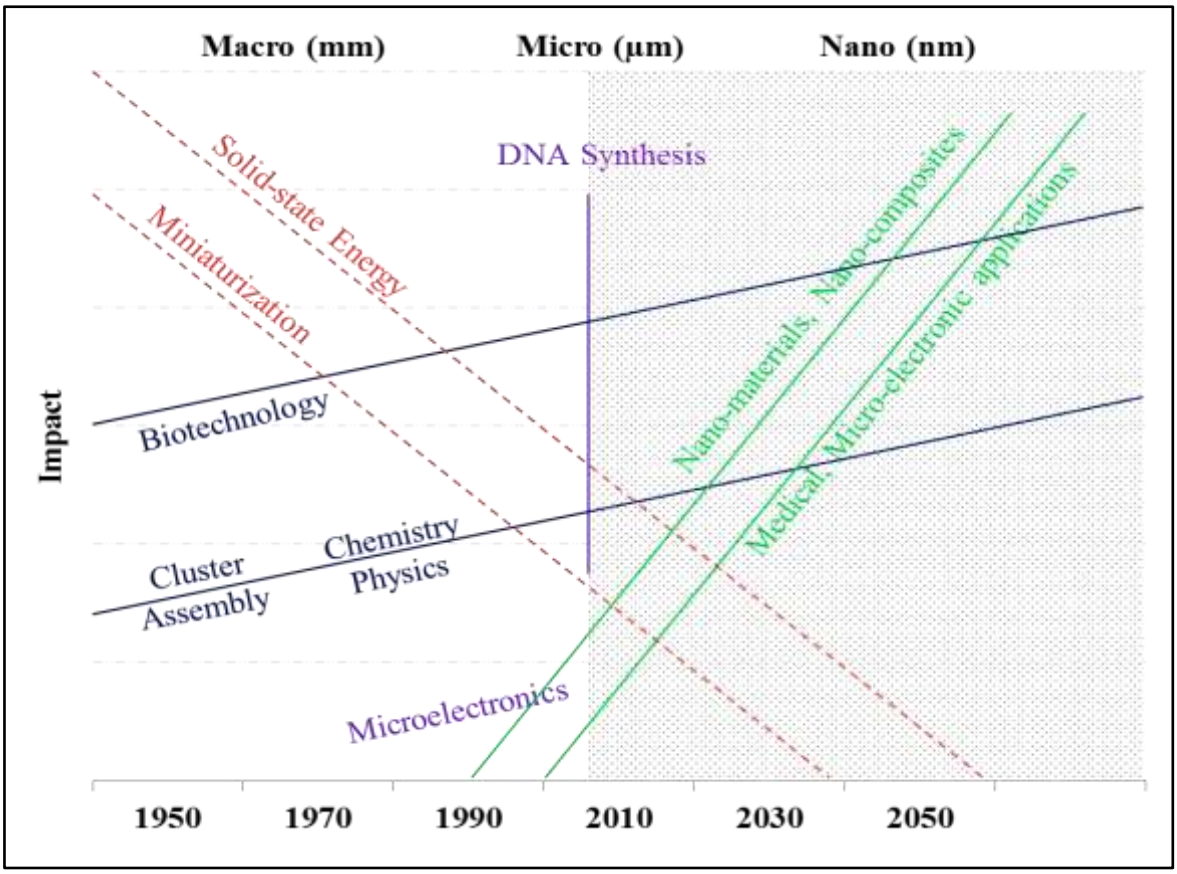


Figure 2.2: Commercial Impact of the nanotechnology [38]

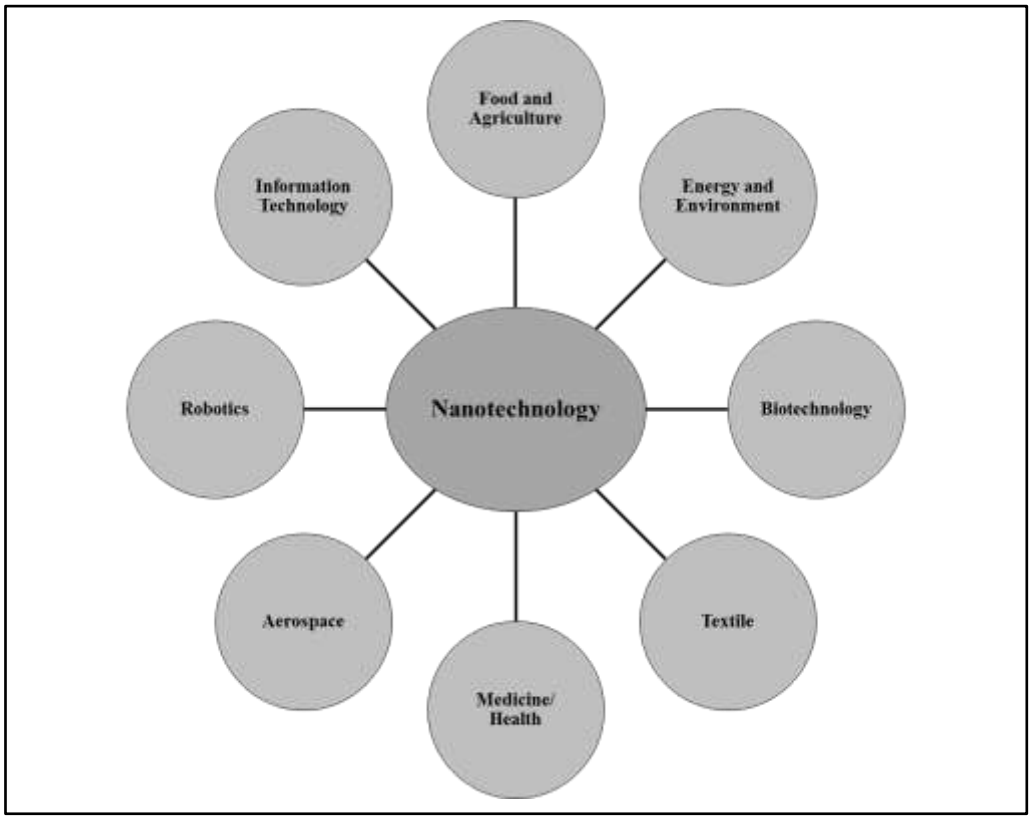


Figure 2.3: Applications of Nanotechnology

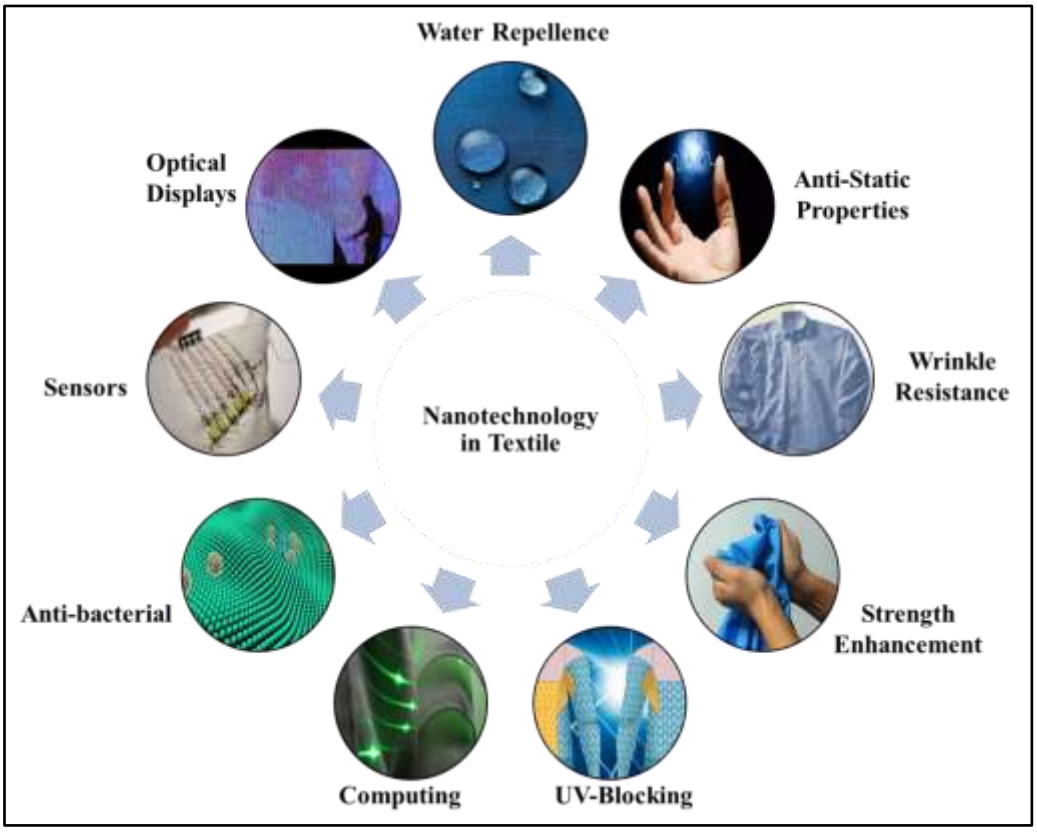


Figure 2.4: Nano-composites/Nanotechnology in Textile

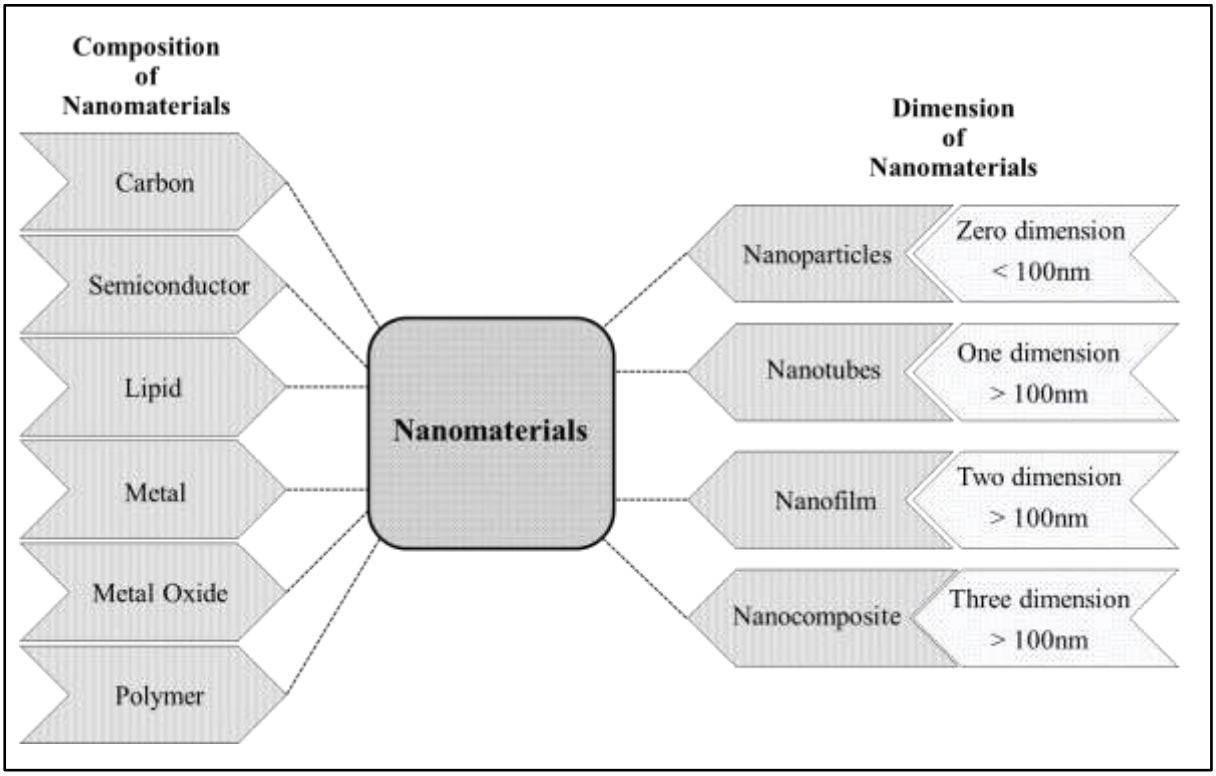


Figure 2.5: Classification of nanomaterials

Nanotechnology in context to textiles:

The textile industry created a quality mark under which a textile material is only be awarded the “nanotechnology” designation if;

a) The nanoparticles or nanostructures are systematically organised to result in a novel function,
b) The nanotechnology employed produces obviously increased functions for the end user without changing the textile characteristics; and
c) The nanoparticles can resist cleaning procedures without harming health or comfort.

This demarcation has been interpreted as the first step taken toward standardising nanotechnology used for textile segment.

Nano Application in Textile:

Nanomaterials and nanotechnology-based techniques are being used at an increasing rate in all sectors of research and technology. The massive textile sector is also not remained untouched in earning the majestic benefits of nanotechnology in a variety of applications. Textile-based nano-composite products are ranging from nano-composite fibres to fabric and extended to intelligent high performance polymeric nano-coatings, they are making inroads not only into high performance advanced applications, but nanoparticles are also being successfully used in conventional textiles to impart new functionality and improved performance. The major advantages of nano technological developments in textiles are increased repeatability, dependability, and resilience [44].

Nano-composite finishing's, in which nanoparticles are distributed in medium and utilised for finishing/coating applications, are a potential path toward developing multifunctional and intelligent high-performance textiles. The nano-composite textiles created with improved and added various functionalities, is the most explored field in producing multifunctional, speciality non-woven. Such non-woven nano-composites are becoming increasingly popular in specialised technological applications such as filter fabric, antibacterial reinforcements, bio-medical applications, tissue engineering, and chemical protection clothing [45].

Nanotechnology has opened up a plethora of new options in the textile industry, resulting in both creative new finishes and novel application processes. By integrating different nanoparticles or generating nanostructured surfaces, it is possible to make chemical

finishing more controlled, durable, and useful. The unprecedented level of textile performance claimed for these nano-finishes, such as antimicrobial, antistatic, stain resistance, UV protective, shrinks proof, and wrinkle resistant abilities, can be used for a variety of technical textile applications, including protective clothing, medical textiles, sportswear, automotive textiles, (Figure 2.4) and so on.

Medical and Healthcare Applications of nanotechnology:

Nanotechnology has already been expanded the range of medical instruments, information, and medical fabrics available to physicians. Nano-material has witnessed the use of nanotechnology in medical to provide precise solutions for illness prevention, diagnosis, and therapy. Few instances about recent inventions took place in this field are listed below:

- Commercial uses have adopted *silver nanoparticles (AgNPs)* as probes for the detection of specific treatments sequences. In this regards the AgNPs are also being studied therapeutically as possible cancer and other illness remedies [46].
- Nanotechnology-enabled imaging and diagnostic tools were opened the path for earlier diagnosis, more customised treatment options, and higher therapeutic success rates [47].
- The use of nanotechnology in the detection and treatment of atherosclerosis, or the accumulation of plaque in arteries, is being investigated. Researchers developed a nanoparticle that mimics the body's "good" cholesterol, known as HDL (high-density lipoprotein), which aids in plaque reduction [48].
- Developments of innovative gene sequencing technologies have allowed single-molecule detection at cheap cost and high speed with a little sample preparation. The related equipment was empowered by the design and fabrication of improved solid-state nano-pore materials [49].
- Researchers in nanotechnology are continuously working on a variety of therapies in which a nanoparticle can encapsulate or otherwise help to deliver medication directly to cancer cells for minimising the risk of injury to healthy tissue. This has the potential to revolutionise cancer treatment and significantly minimise the side-by harmful effects of chemotherapy [50]**.**
- Nanotechnology research for regenerative medicine has included various application areas like bone and neural tissue engineering. Novel materials, for example, can be designed to replicate the crystal mineral structure of human bone or utilised as a

restorative resin for dental applications. Researchers are exploring new techniques to grow complex tissues in the hopes of one day producing human organs for transplant. Researchers are also investigating use of graphene nano-ribbons to aid in the healing of spinal cord damage; preliminary research has indicated that neurons grow well on the conductive graphene surface [51].

- Researchers in nano-medicine are investigating about how nanotechnology can enhance vaccinations and governing for needle-free vaccine as well. Researches are also aimed to develop using this technology a universal vaccine scaffold for the yearly flu vaccine, which would cover more strains while requiring less resources to manufacture each year [52].

2.3.2 CLASSIFICATION OF NANOMATERIALS

Nanomaterials are classified based on their shape, size, and chemical characteristics. The well-known categories of materials defined on the basis of their physical status and chemical composition are illustrated in Figure 2.5.

2.3.2.1 Classification based on composition

These types of nanomaterials are classified based on the component/s utilised in the synthesis process, the composition of nanomaterials and the origin of material/s.

i) Carbon-based Nanomaterials:

The carbon-based nanomaterials are found in a variety of shapes and sizes; hollow tubes or spheres. Carbon nanotubes (CNTs), Fullerenes, and graphene are the good examples of such carbon-based nanomaterials. Chemical vapour deposition (CVD), arc discharge, and laser ablation are normally utilised processes to make carbon-based nanomaterials.

- CNTs have an elongated, tubular shape with a diameter of 1–2 nm. Based on their diameter telicity, they can be anticipated to be conductive like metallic or semiconducting. Physically they seem similar to graphite rolled sheets. The rolled sheets might be having a single, double, or many walls [53].
- Fullerenes represent class of nanomaterials composed of globular hollow cages, such as allotropic carbon forms. They have sparked significant commercial interest because of their exceptional characteristics; electrical conductivity, higher strength, electron affinity, and flexibility [54].

ii) Metal Nanomaterials:

As the name suggests this category of nanomaterials are totally composed of metal precursors, and have distinct opto-electrical capabilities due to their well-known localised surface plasmon resonance (LSPR) features. Metals like Ag, Au, Cu, and Fe, as well as metal oxides like TiO_2, ZnO, and MnO_2, can be used to make these types of nanomaterials. According to Dreaden et al. [55], alkali and noble metal nanomaterials, such as Cu, Ag, and Au, exhibit a wide absorption band in the visible region of the electromagnetic solar spectrum. The regulated synthesis of metal nanomaterials by facet, size, and shape is crucial in today's cutting-edge materials. Metal nanomaterials are used in a variety of scientific fields due to their superior optical characteristics. Just for an example; gold NPs coating is extensively used for SEM sampling to improve the electronic stream, resulting in higher quality SEM pictures [56].

iii) Ceramics Nanomaterials:

Ceramic class of nanomaterials are resourced from inorganic non-metallic solids. Their physical structures can be amorphous, polycrystalline, dense, porous, or hollow, can be used either in heated or cooled or combined conditions. Their preferred usage includes applications such as catalysis, photo-catalysis, dye photo-degradation, and imaging [57].

iv) Semiconductor Nanomaterials:

Semiconductor nanomaterials possess characteristics that are intermediate between metals and non-metals, and as a result, they can cover much wide range of applications in photo-catalysis, photo optics, and electrical devices. According to Hisatomi et al. [58], semiconductor nanomaterials have large band-gaps and hence exhibit considerable changes in characteristics as the band-gap get tuned. As an example, a number of semiconductor nanomaterials have been shown to be extremely effective in water splitting applications due to their appropriate band-gap and band-edge locations. Silicon and ceramic materials are also used to make semiconductor nanoparticles.

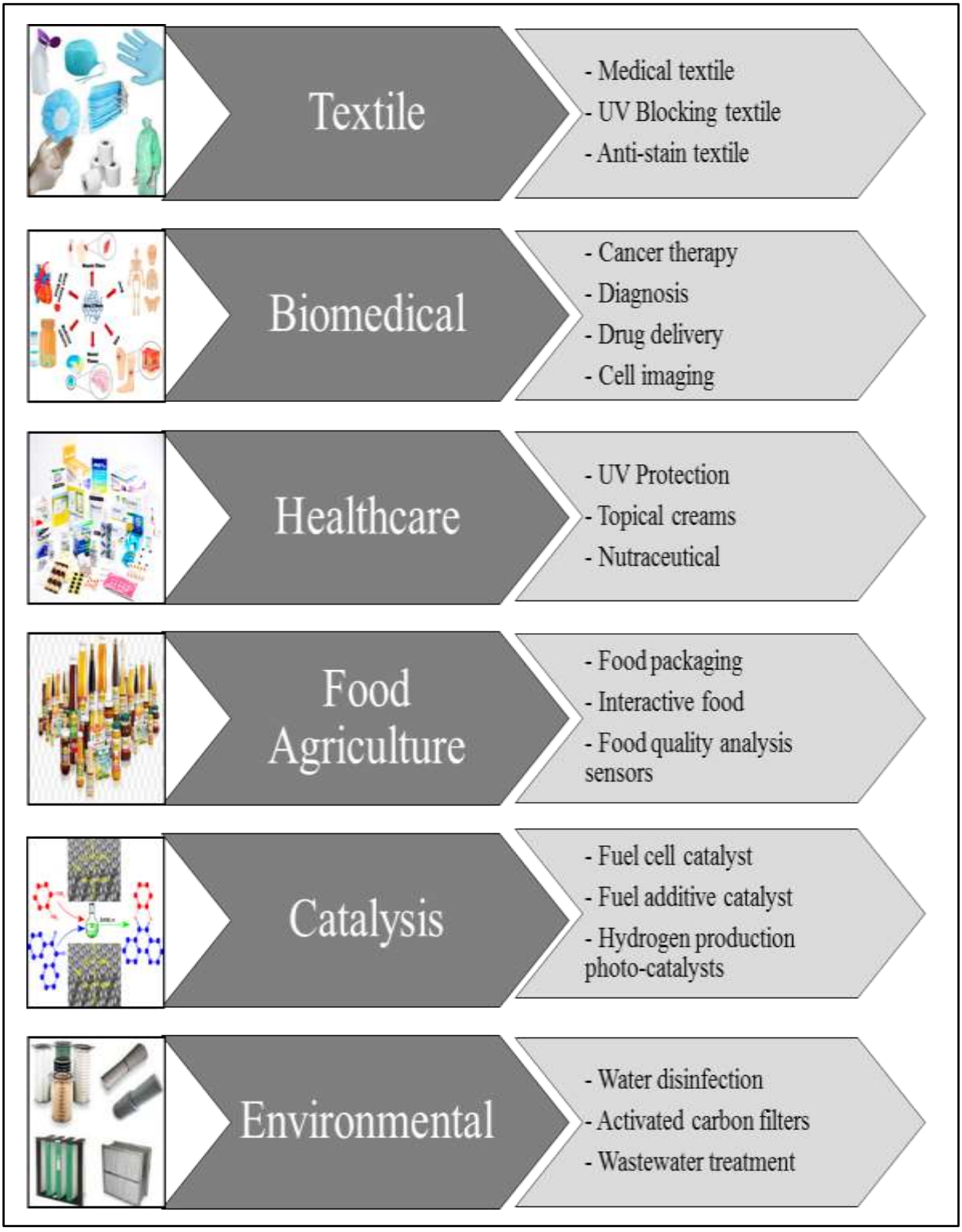


Figure 2.6: Application of silver nanomaterials

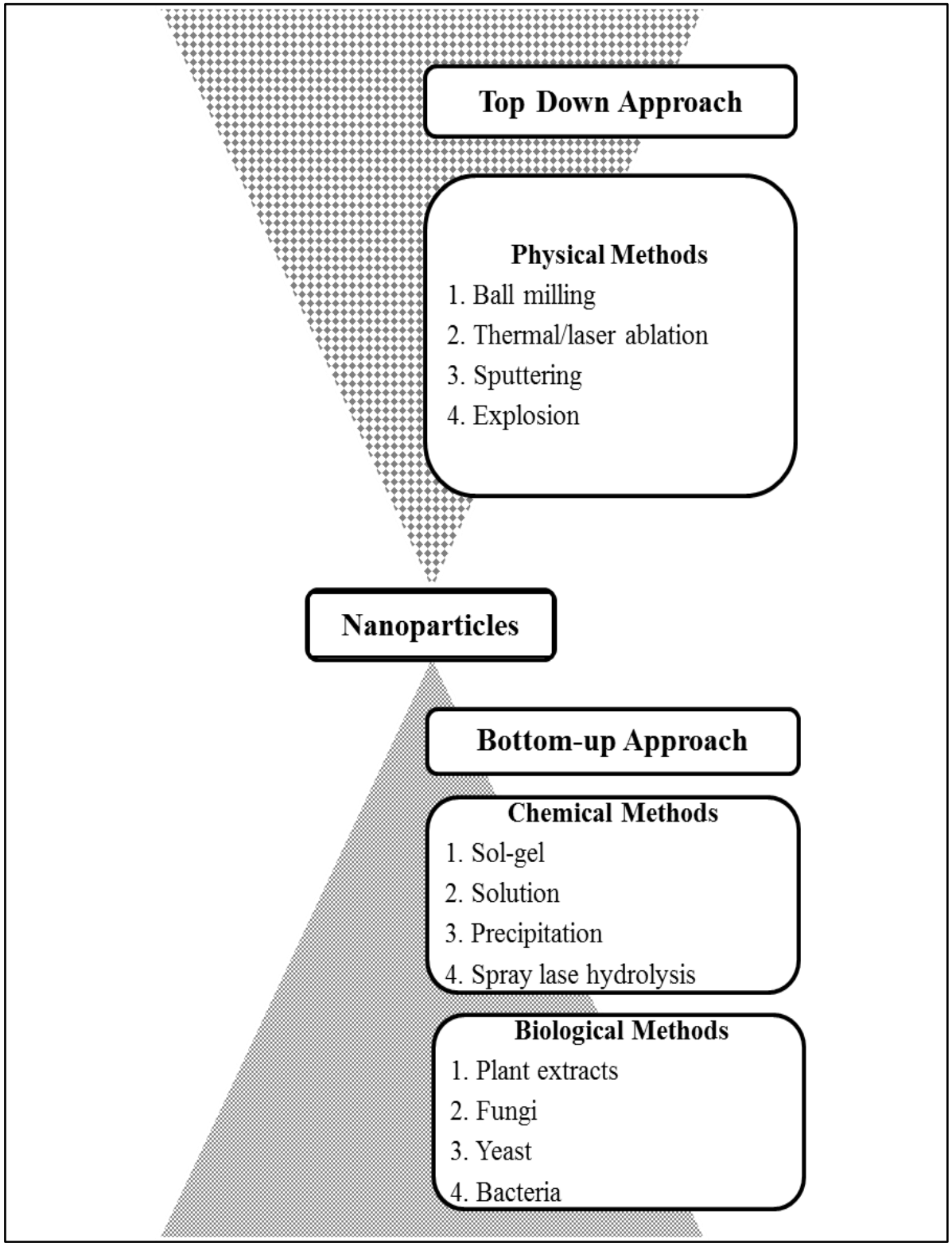


Figure 2.7: Approaches used in Metallic Nanoparticles Synthesis

v) Polymeric Nanomaterials:

Polymer nanoparticles (PNPs) are often regarded as organic-based nanomaterials. They are typically in the shape of nano-spheres or nano-capsules. The nano-spheres are matrix particles with a typically solid overall mass, whereas the other molecules are adsorbed at the outer border of the spherical surface. Conversely in the nano-capsules, the solid mass is fully contained within the particle [59]. The PNPs are readily functionalized and can be found in plenty of applications.

vi) Lipid-based Nanomaterials:

This category refers nanomaterials including lipid moieties and can be used in a variety of biological applications. A lipid nanomaterial is typically spherical, with a diameter ranging from 10 to 1000 nm. According to Gujarati et al. [60], the lipid nanomaterials, contain a solid lipid core and a matrix composed of soluble lipophilic components. The exterior core of these nanomaterials gets stabilised by surfactants or emulsifiers. Lipid nanotechnology is principally a subfield that focuses on the design and production of lipid nanomaterials for different applications such as drug transporters and delivery, as well as RNA release in cancer treatment.

2.3.2.2 Classification based on dimension

Very small nanomaterials can be found in a variety of sizes and shapes, with zero, one, two, and three dimensions. At least one dimension of 1–100 nm should be present in this class of nanomaterials. A variety of morphologies; including tubular, spherical, and irregular, and can be found in single, aggregated, or fused forms for this category of nanomaterials. Nanofibers, nanotubes, quantum dots, and nano-sheets are the most prevalent forms of this type of nanomaterials [61].

i) Zero-Dimensional Nanomaterials:

The most prevalent form of nanomaterial is zero-dimensional nanoparticles, which have all dimensions on the nanoscale (<100 nm) [62]. These nanoparticles are point-like particles, meaning they're around the size of a pinhead. Quantum dots (uniform particle arrays), hollow spheres, nano lenses, and other similar particles are the most widespread examples.

ii) One-Dimensional Nanomaterials:

These nanoparticles have at least one dimension greater than nanoscale (>100 nm), with the remaining dimensions falling within the nano range [62]. Nanofibers, nanotubes, and nanorods are the most common examples for one-dimensional nanoparticles.

iii) Two-Dimension Nanomaterials:

These nanomaterials have two dimensions greater than nanoscale (>100 nm) [62]. Nanofilms, nanolayers, and nanocoating are the most typical instances of this class. Plate-like features characterise this group of nanomaterials.

iv) Three-Dimensional Nanomaterials:

All the three dimensions of this class three-dimensional nanomaterials are greater than 100 nm, although their constituents are smaller (<100 nm) [62]. Nanoscale particles combine to create three-dimensional nanomaterials. These materials are usually nonporous and having a wide range of uses. Nano-composites, nanofiber bundles, and multi-nanolayer structures are well-known examples for the three-dimensional nanomaterials.

2.3.3 SILVER NANOMATERIALS AND ITS APPLICATIONS

According to Sarsar et al. [63], nanosilver exhibits unique or enhanced capabilities based on specific features such as size, distribution, and shape. It has a profound disinfecting effect and used in a variety of applications ranging from traditional remedies to nutritional products. Besides, numerous silver salts and derivatives are commercially produced as antibacterial agents. According to Sarkar et al., silver is harmless for human cells at low quantities but toxic to germs and viruses [64]. Thereby use of nanotechnology to reduce the particle size of materials is an effective and dependable method for enhancing their biocompatibility [65].

Previous researches have shown that nanosilver has demonstrated potential applications in a variety of fields, viz; textiles, bio-medical, healthcare, food and agriculture, catalysts in chemical reactions, environmental applications, electrical batteries and selective coatings for solar energy absorption, as optical elements, in chemical sensing and biosensing,

plasmonics, optoelectronics, biological sensors, and pharmaceutical applications like; their high potential in drug delivery formulations and routes. Their wide span application domains are illustrated in Figure 2.6 [66].

According to Slama et al. [67], as antibiotic-resistant bacterial strains have become a serious concern in public health care, silver as an antibacterial agent is gaining popularity for medicinal purposes. Silver-based medicinal devices, ranging from wound dressings and bandages to coated stents, have been shown to be helpful in suspending and avoiding bacterial infections. Improvements in the creation of innovative silver nanoparticle-containing goods are often pursued. In particular, there is growing interest in the use of silver nanoparticle technology in the creation of bioactive biomaterials, with the goal of integrating the metal's important antibacterial characteristics with the biomaterial's unique performance [68].

2.4 SYNTHESIS OF NANOPARTICLES

Amongst the various options, metallic nanoparticles have been used extensively by the researchers worldwide owing to their superior performance and easy way-out synthesis procedure. They are synthesized either through a top-down or a bottom-up approach, shown schematically in Figure 2.7.

Top-down (Forward) approach: The approach deals with conversion of top macro metallic forms to the bottom nanostructure via physical treatments like; grinding, cutting, spraying or etching out crystal planes. Thereby this method many a times referred as a physical method. The process may be used on a large scale, and it is also capable of achieving nanometre dispersion of one phase in another. This mode's microstructures and phases are frequently thermodynamically metastable [69]. As a result, a top-down method may be thought of as one in which the building blocks are removed from the substrate to produce the nanostructure. This approach is more efficient for producing a large number of nanoparticles with diameters more than 200 nm [70]. Milling, lithographic processes, and machining are examples of top-down synthesis procedures. The primary disadvantage of these techniques is the imperfection of the surface structure. Another drawback of these methods is the significant energy consumption required throughout the preparation to maintain high temperature and pressure [71].

Bottom-up (Reverse) approach: Bottom-up synthesis means that nanostructures are created on the substrate by stacking atoms on top of one other, giving birth to crystal planes. The crystal planes continue to build on top of one another, resulting in the creation of nanostructures. Bottom-up synthesis may thus be conceived of as a synthesis process in which the building blocks are added to the substrate to produce nanostructures. For the production of nanoparticles, the bottom–up method usually employs the wet chemical process [63]. Vapour phase deposition methods, plasma-assisted deposition procedures, molecular beam epitaxy (MBE) and metallorganic vapour phase epitaxy (MOVPE), liquid phase methods, colloidal methods, sol–gel methods, and electro-deposition are all examples of bottom-up synthesis methodologies. In all of these approaches, the atoms of the materials are produced in-situ in a reaction apparatus. These methods yield smaller nanoparticles with a diameter of about 100 nm, albeit at a reduced product amount [70]. However, the widespread usage of harmful and hazardous chemicals is a major source of concern for the environment and living cells [72].

2.4.1 DIFFERENT METHODS USED IN THE SYNTHESIS OF NANOPARTICLES

Nanoparticles have a higher affinity for fabrics due to their enormous surface area to volume ratio and high surface energy. Today's focus is on the advancement and prospective applications of nanotechnology in the creation of multifunctional and smart nano-composite fibres, nanofibers, and other novel nano-finished and nano coated composite textiles for meeting demands of widespread textile application areas [73].

2.4.1.1 Chemical and Physical synthesis methods

The most useful nano synthesis methods in terms of their potential to be scaled up are invariably chemical methods. There are varieties of chemical methods in use to make nanoparticles out of metals (Figure 2.7). Several types of reducing agents can be used to produce nanoparticles; such as sodium triethylborohydride [$Na(C_2H_5)_3BH$], lithium triethylborohydride [$Li(C_2H_5)_3BH$], potassium tartrate ($K_2C_4H_4O_6$), ethylene glycols ($C_2H_6O_2$) and sodium borohydride ($NaBH_4$) [74,75]. For example, nanoparticles from silver nitrate ($AgNO_3$) can be reduced in $NaBH_4$ solution with Tri-sodium citrate ($Na_3C_6H_5O_7$) at room temperature, providing a high yield of Ag^+ nanoparticles [34].

Chemical synthesis is versatile, as can be carried out in all the forms; solid, liquid, or gaseous state. New nano coating processes developed based on physical approaches, such as sol-gel, layer-by-layer, and plasma polymerization etc. can give multi-functionality, intelligence, high durability, and weather resistance to textiles [69].

Although physical methods produce better-defined nanoparticles than chemical procedures, they are more expensive and time-consuming compared to chemical methods, which are cheaper but involve use of dangerous chemicals and solvents as well as evacuate environment harmful gases. Thereby a great deal of interest is shown by researchers in developing an ecologically friendly green process for nanoparticle production. The system makes use of natural resources as reducing and capping agent and eliminates the use of hazardous chemicals and solvents. Thus, in comparison to all the three approaches, bio-based methods employing non-hazardous, eco-friendly plant extracts, fungus, yeast, and bacteria are gaining increasing popularity for the production of nanoparticles [76].

I) Chemical and Physical synthesis of Silver Nanoparticles (AgNPs)

There are a number of different Physical and chemical approaches used to make AgNPs. Ion sputtering, radiolysis, thermal synthesis, ultra-sonication, laser ablation, inert gas condensation, microwave and electrochemical reduction, and sol gel technique are some of the most widely utilised physical and chemical techniques [77].

Many researchers discovered that reducing substances such as sodium borohydride ($NaBH_4$), potassium tartrate ($K_2C_4H_4O_6$), ethylene glycols ($C_2H_6O_2$), ascorbic acid ($C_6H_8O_6$), and others may be utilised to create Ag nanoparticles [78]. For example, Silver nitrate ($AgNO_3$) can be reduced in $NaBH_4$ solution using Tri-sodium citrate ($Na_3C_6H_5O_7$) at room temperature, providing a high yield of AgNPs, which are then agglomerated into oligomeric clusters. These clusters eventually give rise to metallic colloidal silver particles [79]. It is essential to employ protective agents to stabilise dispersive NPs during the production of silver nanoparticles, as well as to protect NPs that can be absorbed on or adhere to nanoparticle surfaces, preventing agglomeration. The inclusion of functional surfactants (e.g., thiols, amines, acids, and alcohols) for interactions with particle surfaces can stabilise particle development and protect particles against sedimentation, agglomeration, and loss of surface characteristics. Polymeric compounds such as poly (vinyl alcohol), poly (vinylpyrrolidone),

poly (ethylene glycol), poly (methacrylic acid), and polymethylmethacrylate have been reported to be the effective protective agents to stabilize NPs [80].

II) Prevalent Chemical and Physical Synthesis Techniques for AgNPs

Commonly used chemical and physical AgNPs syntheses techniques as per cited in the literature are briefly summarised here;

i) Laser ablation: Sylvestre et al. [81], found laser ablation of metallic bulk materials in solution could be used to create silver nanoparticles. Many parameters influence the ablation efficiency and properties of the produced nano-silver particles, including the wavelength of the laser impinging on the metallic target, the duration of the laser pulses (in the femto-, pico-, and nanosecond regimes), the laser fluence, the ablation time duration, and the effective liquid medium, with or without the presence of surfactants [82]. The absence of chemical reagents in solutions is a significant benefit of the laser ablation approach over other methods for producing metal colloids. As a result, this method can produce pure and uncontaminated metal colloids for future uses [83].

ii) UV-initiated photo-reduction: UV-initiated photo-reduction has been described as a simple and successful technique for the production of silver NPs in the presence of citrate, poly vinyl pyrrolidone, poly (acrylic acid), and collagen. According to Huang et al., silver NPs by photo-reducing silver nitrate in layered inorganic laponite clay solutions, acted as a stabilising agent to prevent NPs aggregation. The characteristics of the NPs generated were investigated as a function of UV irradiation time. When exposed to UV light for 3 hours, bimodal size distribution and moderately big silver NPs were produced. Irradiating the silver NPs again fragmented them into smaller sizes using a single distribution mode until a somewhat stable size and size distribution was produced [84]. UV irradiation photo-reduction method at room temperature was used to prepare silver NPs (nanosphere, nanowire, and dendrite) (as protecting and stabilising agent). The concentrations of poly (vinylalcohol) and silver nitrate both played important roles in the development of nanorods and dendrites [85].

iii) Micro-emulsion techniques: Micro-emulsion methods can be used to create uniform and size-controllable silver nanoparticles. The initial spatial separation of reactants (metal precursor and reducing agent) in two immiscible phases is the basis

used for the production of NPs in two-phase aqueous organic systems. The pace of interactions between metal precursors and reducing agents is affected by the interface between the two liquids and the intensity of inter-phase transport between two phases, which is mediated by a quaternary alkyl-ammonium salt. According to Krutyakov et al., metal clusters produced at the interface are stabilised owing to their surface being covered with non-polar aqueous medium stabiliser molecules, and are transported to the organic medium by the inter-phase transporter [86]. One of the primary drawbacks is the usage of very toxic chemical solvents.

iv) Sono-chemical technique: The sono-electrochemistry approach primarily employs ultrasonic power to mechanically move the material. The pulsed sonoelectro-chemical synthetic technique employs alternating sound and electric pulses, and electrolyte composition is critical in shape creation. To prevent aggregation, silver nanospheres can be produced by sono-electrochemical reduction with a complexing agent, nitrilotriacetate [87].

v) Photo-induced reduction: A number of photo-induced or photocatalytic reduction techniques can be used to create silver nanoparticles. Photochemical synthesis is a simple technique with excellent spatial resolution, ease of usage, and adaptability. Furthermore, photochemical synthesis allows one to create NPs in a variety of media such as cells, emulsions, polymer films, surfactant micelles, glasses, and so on. Photo induced reduction was used to create nano-sized silver particles with an average size of 8 nm using poly (styrene sulfonate)/poly (allylamine hydrochloride) polyelectrolyte capsules as micro reactors [88]. Furthermore, it proposed that a photo-induced technique could be utilised to transform silver nano-spheres into triangular silver nano-crystals (nano-prisms) with specified edge lengths in the 30-120 nm range [89]. At room temperature, the direct photo-reduction of $AgNO_3$ in the presence of sodium citrate ($Na_3C_6H_5O_7$) was performed using several light sources (UV, white, blue, cyan, green, and orange). Sato-Berru and colleagues [90] observed that the light-modification process produces a colloid with unique optical characteristics which can be linked to particle size and form.

vi) Microwave-assisted synthesis: Microwave-assisted synthesis is a potential approach for producing silver nanoparticles. When microwave heating outperforms a traditional oil bath, it comes to reliably producing nanostructures with smaller

diameters, narrower size dispersion, and a higher degree of crystallisation. Microwave heating provides faster response times, lower energy consumption, and higher product yields, which also avoids undesirable particle agglomeration [91]. Additional to oil bath elimination, microwave-assisted synthesis in combination with mild reaction media, can substantially minimise chemical wastes and reaction times in a variety of organic syntheses and chemical transformations [92]. Several techniques are used, including commercial microwave ovens, low-cost/low-power ultrasonic cleaners, and two-electrode electro-chemistry.

vii) Irradiation methods: A number of irradiation techniques can be used to create silver nanoparticles. Silver NPs with a well-defined form and size distribution can be produced by laser irradiating an aqueous solution of silver salt and surfactant [93]. A laser was also utilised in a photo-sensitization synthesis technique of producing silver NPs from benzophenone. Low laser powers generated silver NPs of around 20 nm during short irradiation durations, while higher irradiation power produced NPs of about 5 nm. Lasers and mercury lamps can be utilised as light sources for the creation of silver nanoparticles [94].

viii) Electrochemical synthetic method: Silver NPs can be synthesised using an electrochemical technique. Particle size can be controlled by modifying electrolysis settings, and silver NP homogeneity can be improved by changing the composition of electrolytic solutions. Electrochemical reduction at the liquid/liquid interface was used to create polyphenylpyrrole coated silver nanospheroids (3-20 nm). In one of the research the silver metal ion was transferred from the aqueous phase to the organic phase, where it interacted with pyrrole monomer to form this nano-compound [95]. Another work deal with electrochemical reduction within or outside zeolite crystals to create mono-disperse silver nanospheroids (1-18 nm) based on the silver exchange degree of compact zeolite film modified electrodes [96]. Furthermore, an electrochemical technique was used for easy manufacturing of spherical silver NPs (10-20 nm) with narrow size distributions in aqueous solution [97].

ix) Colloidal method: According to Chang et al. [98], chemical synthesis procedures and in those especially colloidal approaches, are very simple and affordable, widely employed for the creation of metal and semiconductor silver nanoparticles. The colloidal techniques rely on the precipitation of nanometre-sized particles within a

continuous fluid solvent matrix to produce a colloidal solution. Unless there is a significant energy barrier to this process, the colloidal material will tend to aggregate owing to attractive van der Waals forces and reduce its energy. The presence and size of an energy barrier to agglomeration will be determined by the particle's attraction and repulsive forces [69]. According to Guerrini et al. [99], by enhancing the repulsive energy, which is typically short range, agglomeration (the development of strong compact aggregates of nanoparticles) and flocculation (the formation of a loose network of particles) may be avoided. This is accomplished by electrostatic or steric stabilisation, both of which result in a repulsive contribution to potential energy. Application of a capping layer to the particle surfaces can provide stabilising steric effects. Additional chemicals are added to the colloidal solution, which attach to the surface of the cluster and block empty coordination sites, limiting further development. These additives can be polymeric surfactants or stabilisers that cling to the surface electrostatically, or anionic capping agents that cling to the cluster covalently [100]. Another option is to precipitate a material within a volume of space specified by a micelle or membrane that serves as a barrier to future development. Following colloidal precipitation on particle surfaces to produce a core–shell nanoparticle structure, deposition on substrates to produce quantum dots, self-assembly on substrates, and finally embedding in other media to form a nano-composite are some of the approaches that can be used to process colloids [101]. Colloidal techniques, which are very simple and affordable, have been widely employed for the manufacture of metal and semiconductor silver nano-crystals through the application of tailored reactions and reaction conditions [98]. One major issue faced with many colloidal techniques is that colloid solutions often age; that is, the particles can grow in size over a time.

III) Brief Summary for Work done on Physical and Chemical synthesis of AgNPs

Polymeric substances such as poly (vinyl alcohol), poly (vinylpyrrolidone), poly (ethylene glycol), poly (methacrylic acid), and polymethylmethacrylate have been derived as excellent NP stabilisers.

Oliveira et al. [102], prepared dodecanethiol-capped silver NPs using the Brust procedure [103] based on a phase transfer of an Au complex from aqueous to organic phase in a two-phase liquid-liquid system, followed by a reduction with sodium borohydride in the

presence of dodecanethiol as a stabilising agent, binding onto the NPs surfaces, avoiding aggregation and making them soluble in certain solvents. Small changes in synthetic variables resulted in significant variations in nanoparticle structure, average size, size distribution width, stability, and self-assembly patterns.

Chen and his colleagues [104], demonstrated the production procedure of monodispersed silver nanoparticles utilising a simple oleylamine-liquid paraffin combination. According to the reports, development process of these NPs can be separated into three stages: growth, incubation, and Oatwald ripening. The higher boiling point of paraffin (300°C) allowed for a wider range of reaction temperatures and thereby good control of the size of silver NPs by adjusting the heating temperature alone without changing the solvent. Furthermore it was concluded that, size of the silver NPs in colloidal could be controlled not only by modifying the heating temperature or the ripening time alone, but also by adjusting the oleylamine-to-silver precursor ratio.

Kim et al. [105], used the polyol method and a modified precursor injection technique to synthesise spherical silver NPs with controlled size and high mono dispersity. The injection rate and reaction temperature were considered as significant parameters in the precursor injection technique for generating uniform-sized silver NPs with a decreased size. At a reaction temperature of 100 °C and an injection rate of 2.5 ml/s, silver NPs with a size of 17 ± 2 nm were produced. Injecting the precursor solution into a hot solution is an efficient way to induce fast nucleation in a short amount of time, resulting in the production of silver NPs with smaller sizes and a narrower size distribution.

Zhang et al. [106], used a highly branched poly (methyl enebisacryl amide amino ethyl piperazine) with terminal dimethylamine groups (HPAMAM-N (CH)) to produce silver colloids. HPAMAM-N (CH) amide moieties, piperazine rings, tertiary amine groups, and hyper-branched structure are critical to its efficient stabilising and reducing properties.

Silver NPs may be prepared at room temperature by simply combining the appropriate metal ions with reduced polyoxometalates, which acted as reducing and stabilising agents. Polyoxometalates are water soluble and conduct stepwise multi-electron redox reactions without disrupting their structure. Illuminating a deaerated solution of polyoxometalate/S/Ag resulted in the formation of silver NPs [107].

IV) Major Disadvantages of physical and chemical methods

As mentioned before, the physical or chemical methods are the most popularly adopted one for the creation of silver nanoparticles. However, a number of publications in the literature show that production of nanoparticles via either of physical or chemical process is indeed expensive, environmentally unfriendly, and costly. As both the synthesis methods necessitate use of both strong and weak chemical reducing agents, as well as protecting agents such as sodium borohydride, sodium citrate, and alcohols [108]. These agents are usually poisonous, combustible, have high energy needs, difficult to dispose of owing to environmental concerns, and also have a low production rate (Figure 2.8) [109].

As a result, there is an increasing need to create ecologically and environment friendly but less expensive techniques for silver nanoparticle production that do not rely on hazardous chemicals in synthesis protocols [110]. Thus, with the increased awareness about environment safety harmful chemicals are now-a-days replaced with natural reagents and system got the new recognition as “Green synthesis”.

Similar to other NPs, biosynthesis techniques for AgNPs have also offered required simple and feasible alternative and established a relatively new and mostly untapped field of research.

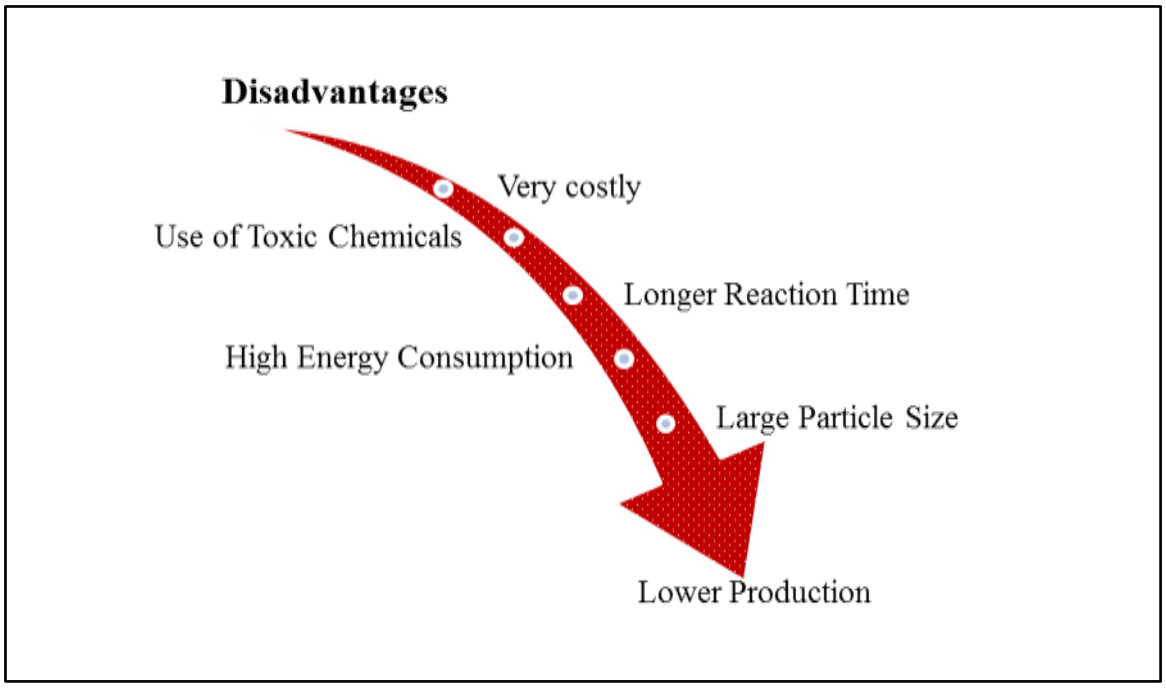


Figure 2.8: Disadvantages of physical and chemical synthesis methods

2.4.1.2 Green Synthesis of Nanoparticles

The goal of green chemistry is to create better, safer chemicals while using the most environmentally friendly and efficient methods of synthesis and waste reduction. Green chemistry attempts to synthesise and utilise chemicals in such a way that they are naturally less harmful to the environment and more efficient to use. Because it focuses on the larger sustainability movement, the terms "green chemistry" and "sustainable" are sometimes used interchangeably [76].

Organisms with nanoparticle production capability range from basic prokaryotic bacterial cells to eukaryotic fungus and plants, used in different domains of green synthesis (Figure 2.7). Just like; bacteria used for gold, silver, cadmium, zinc, magnetite, and iron NPs; yeasts for silver, lead, and cadmium NPs; fungi for gold, silver, and cadmium NPs; algae for silver and gold NPs; and plants for silver, gold, palladium, zinc oxide, platinum, and magnetite NP [111].

Critical aspects of such synthesis include mainly; organism types, inheritable and genetic properties of organisms, optimal conditions for cell growth and enzyme activity, optimal reaction conditions, and selection of the biocatalyst state. Bio-based protocols should be used for the synthesis of highly stable and well-characterized NPs. Some important process parameters should be changed in order to regulate the sizes and morphologies of the NPs, such as substrate concentration, pH, light, temperature, buffer strength, electron donor (e.g., glucose or fructose), biomass and substrate concentration, mixing speed, and exposure duration [112]. Furthermore, the green synthesis of nanoparticles relies greatly on green chemistry. It is broadly speaking chemistry and engineering multidisciplinary area in which chemists and engineers work in coordination to produce chemicals, chemical processes, and commercial items.

It is commonly acknowledged across the world that transitioning to a sustainable society necessitates major adjustments in resource and energy use. Trans-materialization and dematerialization are required to make optimal use of the planet's finite resources. Trans-materialization refers to the process of transitioning from hazardous and non-renewable resources to safer and reusable materials. Dematerialization aims to reduce a society's material and energy inputs while preserving its prosperity.

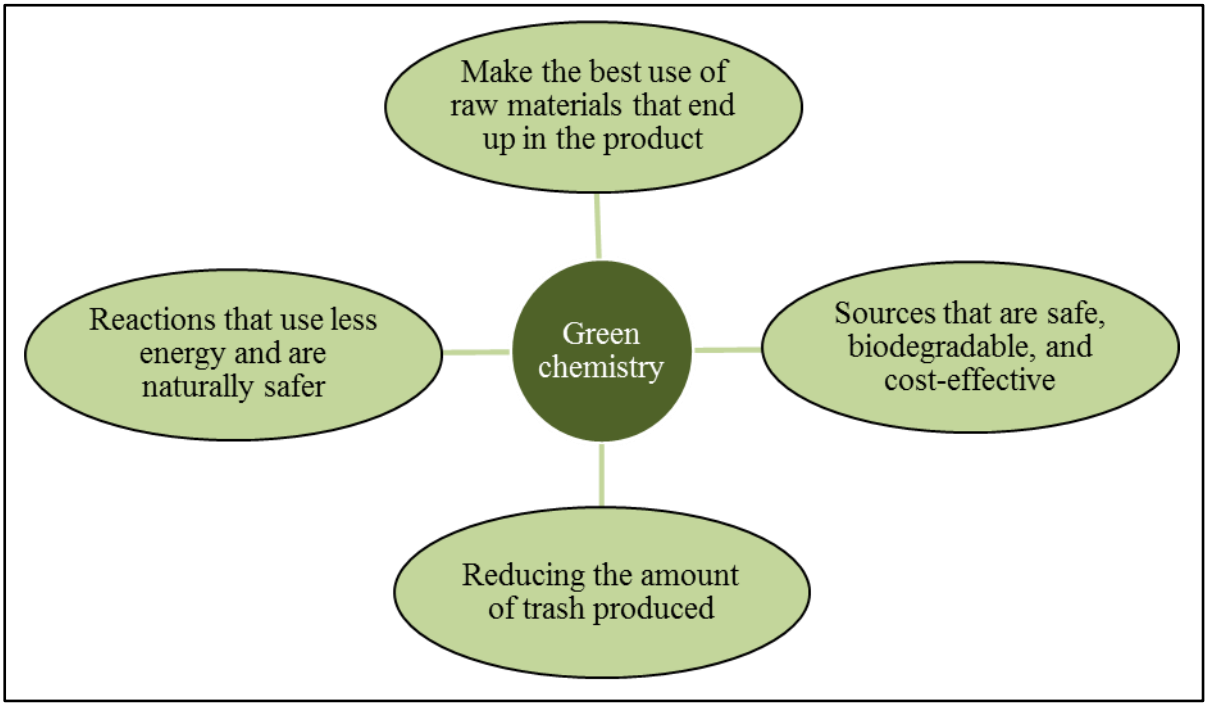


Figure 2.9: Fundamental pillars of green chemistry

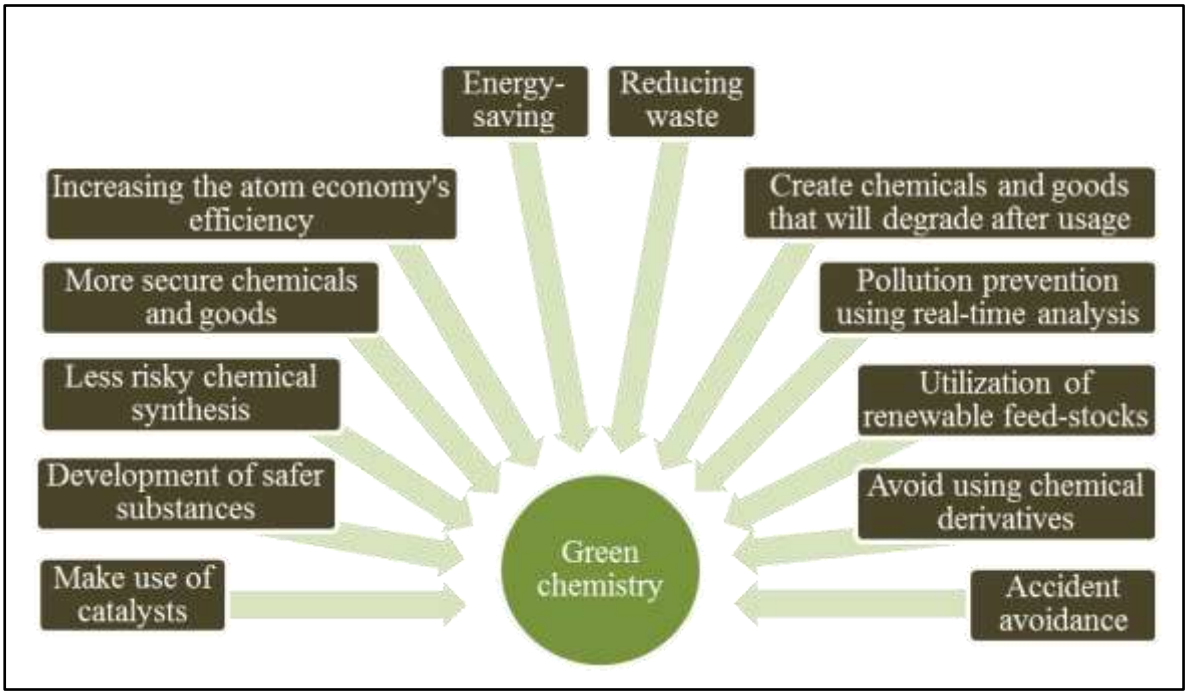


Figure 2.10: Principles of green chemistry

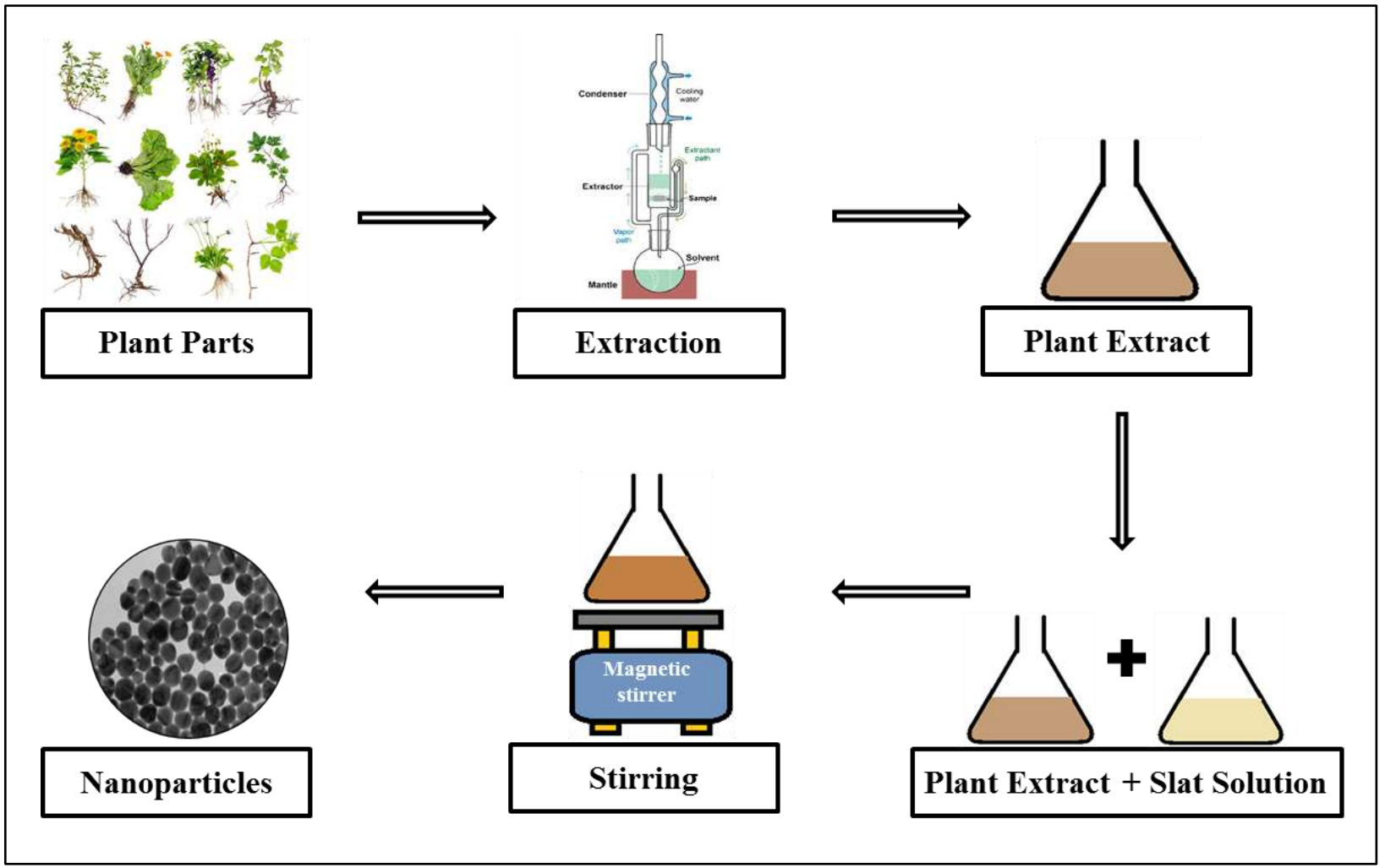


Figure 2.11: Green synthesis of metal nanoparticles

Table 2.2: Green synthesis of nanoparticles from various plant extract/components

Sr. No.	Plant origin	Nanoparticle	Size (nm)	Morphology	Applications	Ref.
1.	Aloe barbadensis Miller (Aloe vera)	Gold and silver	10–30	Spherical, triangular	Cancer hyperthermia, optical coatings	[118]
2.	Aloe barbadensis Miller (Aloe vera)	Indium oxide	5–50	Spherical	Solar cells, gas sensors	[119]
3.	Apiin extracted from henna leave	Silver and gold	39	Spherical, triangular, and quasi-spherical	Hyperthermia of cancer cells and IR-absorbing optical coatings	[120]
4.	Azadirachta indica (neem)	Gold, silver and silver gold alloys	5–35 and 50–100	Spherical, triangular, hexagonal	Remediation of toxic metals	[121]
5.	Calotropis gigantean (Milkweed)	zinc oxide	1.5–11	Spherical	-	[122]
6.	Coriandrum sativum (coriander)	Gold	6.75–57.91	Spherical, triangular, truncated triangular, decahedral	Drug delivery, tissue/tumor imaging, photothermal therapy	[123]
7.	Cymbopogon flexuosus (lemongrass)	Gold	200–500	Spherical, triangular	Infrared-absorbing optical coatings	[124]
8.	Ocimum sanctum (tulsi; root extract)	Gold and silver	30 and 10–20	Crystalline, hexagonal, triangular and spherical	Biolabeling, biosensor	[125]
9.	Tanacetum vulgare (tansy fruit)	Gold and silver	11, 16	Triangular, spherical	Antibacterial, sensors	[126]
10.	Terminalia catappa (almond)	Gold	10–35	Spherical	Bio-medical field	[127]

Table 2.3: Advantage of green synthesis of NPs from plant parts

Omnipresent:	Plants are everywhere because they are a rich source of biomolecules and are readily available throughout the earth's surface in large quantities.
Simple Reaction:	The reaction is carried out without the need of high temperatures, pressures, or energy.
Rapid reaction rate:	When compared to other physicochemical methods, the reaction rate is relatively fast.
Use of non-toxic chemicals:	No hazardous chemicals are required for synthesis.
Reducing and stabilising agent:	The plant extract acts as a reducing and stabilising agent.
Free from hazardous by-products:	When compared to physiochemical techniques, the end product is free of toxic by-products.
Environmentally safer:	As-synthesised nanoparticles are less harmful to the environment when used in bio-medical applications.

Green chemistry is gaining popularity and is desperately needed as a result of global concerns related with environmental pollution. Hence it is the environment friendly way to produce materials with reduced usage and generation of hazardous substances from biodegradable, safe materials, and while not completely, a few issues associated with nano-toxicology and hazards can be dealt with to a good extent with green synthesis approaches. “Green synthesis” is a new platform for developing innovative products that benefit human and environmental health, and it has the potential to transform large-scale nano synthesis methods [113].

These green nanomaterial synthesis techniques are expected to help the environmental and bio-medical segments of nanotechnology applications in the future. This novel approach can be regarded as a model for clean and sustainable nanomaterials. The fundamental pillars of green chemistry are shown in Figure 2.9.

Green synthesis technologies minimise the need for hazardous chemicals while increasing efficiency in producing the required quantity of pure material in a cost-effective way. It also offers potential design strategies for ensuring that the nanoparticles created are effectively safer by assessing biological and environmental risks.

According to Tundo et al. [114], green chemistry is based on twelve concepts that have been applied to the study of chemical compounds and synthetic processes. The primary goals of these principles (Figure 2.10) are to reduce chemical toxicity and waste in order to avoid contamination.

The green synthesis of metal nanoparticles is a unique, ecologically friendly, and active topic of study in which metal nanoparticles are produced using bacteria, fungi, and plants.

Plant extract was utilised in the production of the inorganic nanomaterials based on this method. Plant-mediate green production of metal nanoparticles is a rapid, cost-effective method that yields very stable nanoparticles. It is a one-pot synthetic method that is environmentally friendly and safe for human therapeutic usage. Plant-based procedures, as opposed to conventional synthesis methods, satisfy all of the requirements for green synthesis. These methods are energy efficient and time efficient, as well as acceptable for the environment, i.e. eco-friendly.

The bio-fabrication of metal NPs makes an important addition to nanomaterials research by stressing the possibilities of green chemistry routes to manufacture technically significant nanomaterials [115]. To summarise, the green synthesis of metal NPs is comprised of three critical stages.

i. Selecting a non-hazardous, biocompatible solvent medium.
ii. Selection of environmentally acceptable reducing agents.
iii. Selection of non-hazardous stabilising and size-controlling agents

Green synthesis of Nanoparticles by using Plants: The green synthesis of nanoparticles takes a "bottom-up" strategy, relying mostly on chemical and biological processes of production. Since ancient times, many plant components such as the root, stem, leaf, latex, seed, and so on have been utilised as therapeutic herbs. At present, biological approaches for nanoparticle production have primarily relied on plants. Plants include several types of antibacterial chemicals, viz; phenolics, terpenoids, alkaloids, lectins, polypeptides, and flavonoids [116].

According to Jain et al. [117], the medicinal plants with high phenolic content have gained popularity due to their powerful antioxidant effects. Such self-sufficient plant components have recently emerged as possible options for green production of metal nanoparticles. Table 2.2 summarizes the green synthesis of nanoparticles from several plant extracts/components practiced in various applications.

The green production of nanoparticles from various plant components has found wide publication coverage in present era. The initial stage in synthesis is extraction from plant components, which may be accomplished by a variety of techniques including as maceration, infusion, digesting, and decoction. A common technique of extraction from plants is hot continuous extraction using a Soxhlet device [128].

Plant extract is mixed with a metal salt solution at room temperature to create nanoparticles from plant components. The reaction is finished in a short interval of time. Plant extracts have been used effectively in manufacturing nanoparticles of silver, gold, iron, copper, palladium, platinum, and many other metals [129]. Figure 2.11 depicts a general approach for green metal nanoparticle production. There are several benefits realized with green approach of nano synthesis, major rewards are concise in Table 2.3.

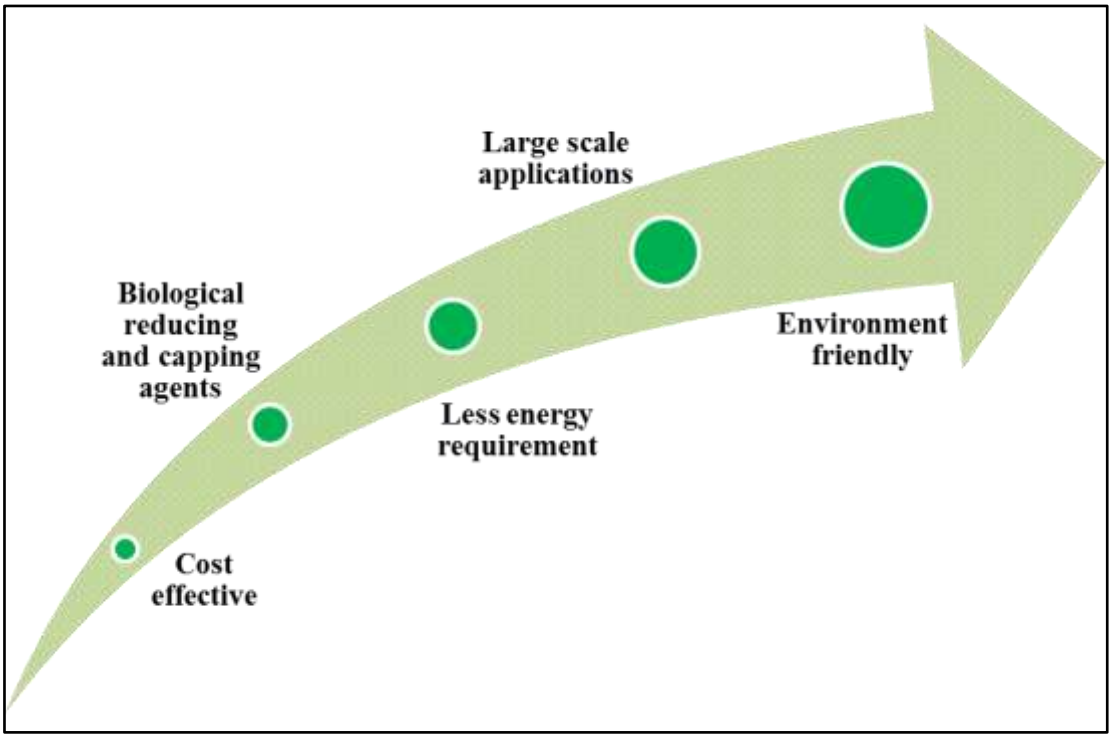


Figure 2.12: Favourable features of Green synthesis approach for Silver nanoparticles

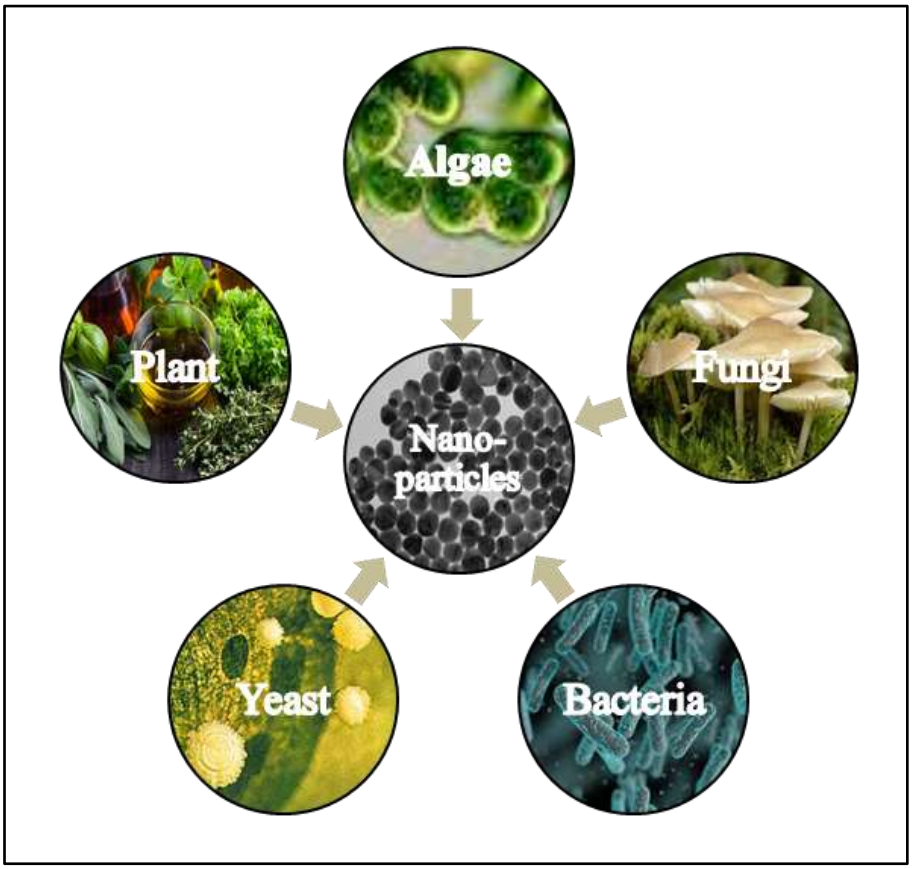


Figure 2.13: Green mediums used in AgNPs synthesis

I) Green synthesis of Silver nanoparticles (AgNPs)

The biosynthesis is employing organic particles obtained from plant sources as concentrates outperforms chemical techniques thereby this approach is also known as "Green synthesis". It is non-toxic, leaving no polluting effects on the environment and uses safe reagents, which are neither dangerous nor harmful to any living organisms. Hence, biological production of nanoparticles is a one-step bio-reduction approach it requires less energy [130]. Thus, utilization of cheaper abandoned natural resources and consumption of very less costly energy, makes system as a good economical and environ-friendly alternative to physical and chemical approaches (Figure 2.12).

Many green/biological mediums have been worked out by various researchers in synthesis; they are depicted in Figure 2.13. The major classes of resources used in the course of investigation are algae, bacteria [131,132], fungi, yeast [133-136], and plants [137-142]. A brief mention about some important findings observed in different categories is given below for reference.

i) Algae

There are a few studies reported on algae species based AgNPs synthesis. The little work carried out incorporates use of marine algae like; cyanobacteria, as biological reagents. Chaetoceros calcitrans, Chlorella salina, Isochrysis galbana, and Tetraselmis gracilis [143].

Oscillatoria willei NTDM01, a marine cyanobacterium, was utilised in the production of silver nanoparticles of 100-200nm. The desired NPs were obtained after 72 hours of incubation of silver nitrate solution with washed marine cyanobacteria, turning the solution yellow. Extracellular production of spherical silver NPs of 7-16 nm was observed after 120 hours of exposure to 10 M aqueous $AgNO_3$ in Spirulina platensis biomass at 37°C and pH 5.6. Proteins expectedly involved for NP reduction and stability in this course of synthesis. In the instance of C. vulgaris, the proteins in the extract performed both Ag ion reduction and shape regulation for NP synthesis. The Ag nano plates were obtained at room temperature. The hydroxyl groups in Tyr residues and the carboxyl groups in Asp/Glu residues reduced Ag ions. This caused anisotropic development of Ag nanoplates, resulted in rod-like particles with a mean length of 44 nm and width of 16-24 nm [144].

ii) Bacteria

Klaus et al. [145], were the first to employ bacteria Pseudomonas stutzeri to manufacture silver nanocrystals with a size of 200 nm. This has encouraged utilisation of alternative strains, such as Aspergillus flavus and Trichoderma, in manufacturing silver nanoparticles.

Pseudomonas stutzeri AG259 effectively produced silver nano-crystals with various compositions [146]. P. stutzeri AG259, a silver-resistant bacterial strain isolated from silver mine, accumulated silver NPs and silver sulphide intra-cellularly, with sizes ranging from 35 to 46 nm [147]. When P. stutzeri AG259 was challenged with high concentrations of silver ions during culture, larger particles were produced, resulting in intracellular production of silver NPs ranging in size from a few nm to 200 nm [148]. P. stutzeri AG259 detoxified silver via periplasmic precipitation and reduction to elemental silver with a variety of crystal typologies, such as hexagons and equilateral triangles, as well as three different types of particles: elemental crystalline silver, monoclinic silver sulphide acanthite (Ag S), and a further undetermined structure. The periplasmic space limited crystal thickness but not breadth, which may be rather considerable (100-200 nm).

Highly stable silver NPs (40 nm) were produced through bio-reduction of aqueous silver ions with a culture supernatant of a non-pathogenic bacteria, Bacillus licheniformis [149]. Even, well-dispersed silver nano-crystals (50 nm), important for uniform NPs distribution were observed while working with the bacteria B. licheniformis [150].

Another work showed the fast manufacture of metallic silver NPs by the reduction of aqueous Ag ions by culture supernatants of Klebsiella pneumonia, Escherichia coli, and Enterobacter cloacae [143]. The synthetic process was quite quick, with silver NPs produced within 5 minutes of silver ions coming into contact with the cell filtrate. It appears that nitro-reductive enzymes are responsible for silver ion bio-reduction.

iii) Fungi

Extra-cellularly silver nanoparticles of size 5-50 nm were produced with Fusarium oxysporum, with no indication of flocculation of the particles even a month after the reaction [151]. The long-term stability of the nanoparticle solution observed was expectedly caused due to the protein stabilisation of the silver particles. Morphology of the NPs varied greatly, with typically spherical and occasionally triangular forms has been seen in the micrographs. Silver nanoparticles have shown a significant binding with proteins such as cytochrome c. (Cc). This protein has the potential to self-assemble on a citrate-reduced silver colloid surface [152]. Adsorption of (Cc)-coated colloidal gold NPs onto aggregated colloidal Ag resulted in the formation of an Ag: Cc: Au nanoparticle conjugates [153]. Appearance of an absorption band at around 270 nm in UV-vis spectra from the reaction mixture after 72 hours' time gap was attributed to electronic excitations in tryptophan and tyrosine residues in the proteins.

Jebali et al. [154], successfully used Geotricum species to manufacture Ag nanoparticles. Geotricum was cultivated on Sabro Dextrose Agar (SDA) medium for 96 hours at 25 ± 1 °C. Silver nitrate solution was converted into nanosilver using mycelium. These funguses were used to extracellularly manufacture silver nanoparticles. This fast, eco-friendly, and straightforward synthetic technique may be utilised to create Ag nanoparticles with diameters ranging from 30 to 50 nm. This approach is considered to be environmentally benign and cheap cost due to the utilisation of room temperature settings and the lack of hazardous reducing chemicals.

Kumar and colleagues [155], used -NADPH dependent nitrate reductase isolated from F. oxysporum and phytochelatin to get vitro enzymatic production of silver NPs with various chemical compositions, sizes, and morphologies. The silver ions were reduced in the presence of nitrate reductase, and resulted in the creation of a stable silver hydrosol 10-25 nm in diameter which were stabilised by the capping peptide. The use of a particular enzyme in the in vitro production of NPs demonstrated intriguing benefits. When these NPs were used in homogeneous catalysis and other applications such as non-linear optics, eliminated the need for downstream processing. The most significant benefit of this procedure based on purified enzyme was the creation of a novel technique for green synthesis of

nanomaterials with a wide variety of chemical compositions and forms without aggregation.

Kathiresan and colleagues [156], demonstrated that spherical silver NPs could be generated when the culture filtrate of Penicillium fellutanum was treated with silver ions and kept in the dark. Penicillium sp. J3 isolated from soil was found capable enough for producing silver nanoparticles [157]. The bio-reduction of silver ions on the surface of cells and proteins played an important role in the synthesis and stability of the produced NPs [158].

iv) Yeast

Yeasts are unicellular microorganisms that reproduce by an asymmetric cell division process known as budding and are classified as either Ascomycetes (Saccharomyces and Candida) or Basidiomycetes (Filobasidiella and Rhodotorula) [159]. Yeasts have an innate capacity to absorb and accumulate high quantities of harmful metal ions from their surroundings, and they may adjust to this stress by employing diverse detoxifying processes such as mobilisation, immobilisation, or metals transformation. These yeast bioremediation processes is important in the green synthesis of NPs [160]. NPs biosynthesis can be intracellular or extracellular, depending on the yeast species [161].

Many yeast species have been utilised in the production of AgNPs, including Saccharomyces cerevisiae, Saccharomyces boulardii, Candida utilis NCIM 3469, Candida lusitaniae, silver-tolerant yeast strain MKY3 and a marine yeast Yarrowia lipolytica strain [162]. Elahian et al. [163], used a genetically engineered strain of Pichia pastoris for AgNP production in their recent research. Extremophilic yeasts isolated from acid mine drainage have also been seen to be capable of producing AgNPs [164].

According to Fernandez et al. [165], antifungal activity of AgNPs synthesised using two epiphytic yeasts isolated from apple peel, Cryptococcus laurentii and Rhodotorula glutinis, and its potential application as an efficacious nano-fungicide against phyto-pathogenic fungi that cause postharvest diseases in pome fruits. Because epiphytic yeasts, such as C. laurentii and R. glutinis, are considered GRAS

(Generally Recognized as Safe) microorganisms, NPs generation utilising these two yeasts offers considerable benefits in agroecosystem applications [166].

v) Plants

Plant-based NPs synthesis is extremely cost efficient and hence preferentially utilised as an economical and beneficial option for large-scale AgNPs production [167]. A variety of plant leave are used till date for the green synthesis of silver nanoparticles, few are summarized in Table 2.4.

Wide caders of plant leave were used in the synthesis of silver nanoparticles embrace: Acalypha indica, Anacardium occidentale, Aloe barbadensis, Aloe ferox, Azadirachta indica, Camellia sinensis, *Calotropis procera*, Capsicum annuum, Cinnamomum camphora, Chenopodium album, Cochlospermum gossypium, Coleus amboinicus, Coriandrum sativum, Clerodendrum inerme, Diospyros kaki, Enhydra fluctuans, Eucalyptus hybrid, Eclipta, Elaeis guineensis, Garcinia mangostana, Ginko biloba, Gliricidia sepium, Hevea brasiliensis, Hibiscus rosa sinensis, Lawsonia inermis, Ludwigia adscendens, Lippia citriodora, Magnolia kobus, Mentha piperita, Murraya koenigii, Moringa oleifera, Nelumbo nucifera, Ocimum sanctum, Phyllanthus amarus, Pinus desiflora, Parthenium hysterophorus, Pelargonium graveolens, Platanus orientalis, Syzygium aromaticum, Swietenia mahogany, Sesuvium portulacastrum, Svensonia hyderabadensis, and Tanacetum vulgare [174].

The stem of *Calotropis procera*, Desmodium triflorum, Vitex negundo, and fruits of Allium cepa, Citrus limon, Helianthus annuus, Solanum lycopersicum, and Sorbus aucuparia were also used for the eco-friendly synthesis of silver nanoparticles [174]. Table 2.5 summarises the results of some notable plants-mediated green synthesis carried out for AgNPs.

Table 2.4: Various medicinal plant used for nanoparticles formation with their therapeutic uses

Sr. No.	Plant Description	Therapeutic uses	Ref.
1.	 Botanical name: *Calotropis procera* Family: Apocynaceae Vernacular name: Milkweed Part used: Roots, *leafs*, fruits	Anti-Cancerous, Anti-Bacterial, Anti-Inflammatory, Anti-Diarrhoeal, Anti-Pyretic, Anti-Oxidant, Wound Healing, etc.	[168]
2.	 Botanical name: Swertia chiryta Family: Gentianaceae Vernacular name: Chirayat Part used: Stem	Antipyretic, analgesic, skin care, cures anaemia, anti-cancer etc.	[169]
3.	 Botanical name: Terminalia belerica Family: Combretaceae Vernacular name: Bahera Part used: Fruit	Expectorant, heart remedy, antiseptic, cardiovascular disease, diarrhea, constipation, cough, etc.	[170]

4.		Botanical name: Withania somnifera Family: Solanaceae Vernacular name: Ashwagandha Part used: Roots, leafs, fruits	Anti-inflammatory, hepatoprotective, antibacterial, antitumour, diuretic, ntiarthritic, etc.	[171]
5.		Botanical name: Hypericum perforatum Family: Hypericaceae Vernacular name: Goatweed Part used: Roots, leafs, flowers, fruits	Antibacterial, Antibiotic injections, antioxidant, etc.	[172]
6.		Botanical name: Lantana camara Family: Verbenaceae Vernacular name: Indradhanu, Ghaneri Part used: Plant roots, leafs, flowers	Convulsions, malaria, reatment of wounds, skin diseases, antioxidants, antipyretics, swelling, etc.	[173]

Table 2.5: Green synthesis of AgNPs from various plant extract/components

Sr. No.	Plant origin	Size (nm)	Morphology	Applications	Ref.
1.	Acalypha indica	20–30	Spherical	Antibacterial activity against water borne pathogens	[175]
2.	Brassica juncea (mustard)	2–35	Spherical	Phytoremediation, Hyperaccumulators	[142]
3.	Calotropis gigantean (Milkweed)	11–84	Spherical	Antimicrobial	[176]
4.	*Calotropis procera (Milkweed)*	20-30	Spherical	Antibacterial	[177]
5.	Carica papaya (papaya)	60–80	Spherical	Aging skin, Wound healing	[178]
6.	Citrus limon (lemon)	< 50	Spherical, spheroidal	Skin care, Antioxidants	[179]
7.	Eucalyptus citriodora (neelagiri)	20	Spherical	Antibacterial	[180]
8.	Eucalyptus hybrida (safeda)	50–150	Crystalline, spherical	Skin ointment, Antiseptics	[181]
9.	Garcinia mangostana (mangosteen)	35	Spherical	Antimicrobial activity against E. coli and S. aureus	[182]
10.	Jatropha curcas (seed extract)	15–50	Spherical	Antimicrobial	[183]
11.	Ludwigia adscendens (ludwigia)	100–400	Spherical	Antiseptic	[184]
12.	Mentha piperita (peppermint)	5–30	Spherical	Antimicrobial	[185]
13.	Morus (mulberry)	15–20	Spherical	Antimicrobial activity against E. coli and S. aureus	[186]
14.	Nelumbo nucifera (lotus)	25–80	Spherical, triangular, truncated triangular, decahedral	Larvicidal activity against malaria and filariasis vectors	[187]
15.	Ocimum sanctum (tulsi; root extract)	10 ± 2 and 5 ± 1.5 nm	Spherical	Catalytic reduction	[188]

II) Brief summary on AgNPs synthesis by using plants

Camellia sinensis (green tea) extract has been utilised in the biosynthesis of silver nanoparticles as a reducing and stabilising agent [189]. C. sinensis extract included phenolic acid-type biomolecules (e.g., caffeine and theophylline) that appeared to be responsible for the synthesis and stability of silver NPs. Black tea leaf extracts were also utilised in the manufacture of silver nanoparticles [190]. The NPs were stable and came in a variety of forms, including spheres, trapezoids, prisms, and rods. Polyphenols and flavonoids appeared to be involved in the production of these NPs.

Green plants extracts i.e. alfalfa (Medicago sativa), lemongrass (Cymbopogon flexuosus), and geranium (Pelargonium graveolens) have been served as reactants in the production of silver nanoparticles. Furthermore, by confronting silver ions with Datura metel leaf extract, a large density of very stable silver NPs (16-40 nm) was rapidly produced. This plant's leaf extracts include biomolecules such as alkaloids, proteins/enzymes, amino acids, alcoholic compounds, and polysaccharides that might be utilised as reducing agents to react with silver ions and therefore used as scaffolds to control the production of silver NPs in solution [191].

Capsicum annuum can be used to green synthesise silver nanoparticles. Proteins containing amine groups inhibited the formation of silver ions and served as a control during synthesis. The protein's secondary structure was discovered to be changed after interaction with the silver ions. With increasing reaction time, the NPs' crystalline phase changed from polycrystalline to single crystalline and their size grew. To describe the probable production process of silver NPs in Capsicum annuum L extract, a recognition-reduction restricted nucleation and growth model was proposed [192].

Euphorbia hirta leaf extract was used to create spherical silver nanoparticles (40-50 nm) [193]. These nanoparticles exhibited antibacterial activity against Bacillus cereus and Staphylococcus aureus. Within 30 minutes, Acalypha indica (Euphorbiaceae) leaf extracts generated silver NPs (20-30 nm) [194]. These nanoparticles shown good antibacterial action against waterborne pathogens, E. coli, and V. cholera (minimum inhibitory concentration (MIC) =10 g/ml). Furthermore, when 10 ml of Moringa oleifera leaf extract was combined with 90 ml of 1 mM aqueous $AgNO_3$ and heated at 60-80°C for 20 minutes, silver NPs (57 nm) were generated. These nanoparticles demonstrated significant antimicrobial activity

against pathogenic microorganisms such as Staphylococcus aureus, Candida tropicalis, Klebsiella pneumoniae, and Candida krusei [195].

The use of Cacumen platycladi extract in the green production of silver nanoparticles was explored. Reducing sugars and flavonoids in the extract appeared to be primarily responsible for silver ion reduction, and their reductive capability was enhanced at 90°C, resulting in the production of silver NPs (18.4 – 4.6 nm) with a restricted size distribution. The NPs generated were antibacterial active against both gram negative and gram positive bacteria (E. coli and S. aureus) [196]. The bark extract of Cinnamon zeylanicum might be utilised in the biosynthesis of cubic and hexagonal silver nanocrystals (31–40 nm) [197].

Kasthuri and colleagues [198], demonstrated that apiin (Natural flavonoid) isolated from henna plants may generate anisotropic gold and quasi-spherical silver NPs. Apiin's secondary hydroxyl and carbonyl groups were responsible for metal salt bio-reduction. They employed varying quantities of apiin to alter the size and shape of the NPs (as a reducing agent). The NPs were stable in water for three months, which might be ascribed to apiin surface binding to the NPs. Furthermore, geraniol, a volatile molecule derived from P. graveolens, was utilised in the manufacture of silver nanoparticles (1–10 nm) [199].

Polyethylene glycol, polyvinyl pyrrolidone, starch, heparin, poly-cationic chitosan, sodium alginate, and gum acacia have all been described as reducing and stabilising agents for silver NP production. For example, it was found that gum kondagogu (non-toxic polysaccharide produced as an exudate from the bark of Cochlospermum gossypium) may be used to manufacture mono-disperse spherical silver NPs (3 nm). Carboxylate and hydroxyl groups were thought to be important in the complexation and bio-reduction of silver ions into NPs. Because the gum acts as a matrix for both bio-reduction and the stability of the produced NPs, this approach proved consistent with green chemistry principles. Because of the inexpensive cost of plant-derived biopolymer, this technique might be used for large-scale production of very stable mono-dispersed NPs [200].

III) Calotropis (Milkweed) as a Medicinal Herbs

The word "Calotropis" is derived from Greek and means "beautiful boat keel." Calotropis has historically been of commercial importance due to the fact that various sections of the plant are useful. Many Hindus see it as a holy plant, linked with the observances of the maruts (storm/winds deities), who have been greeted with a garland of

flowers of Calotropis on Saturdays for millennia. Calotropis was connected with sun-worship in ancient times in the Arabian world, and the leave of Calotropis, in the form of arkapattra (sun-leaf) or arkaparna (lightning leaf), were also associated with sun worship in Vedic times. In general, the Calotropis species, include more than 140 species of plants, have long been included in traditional medicine and have been named after the Greek god of healing [201]. From the various species of Calotropis, Calotropis procera (CP) is known as 'Raktha Arka', while Calotropis gigantea is known as 'Arka kalpna' and 'Sweta Arka', according to the Aryuvedic classics [202]. Both species are distributed all over the world, however the 'Calotropis procera' has purple flowers and is easily available, and the 'Calotropis gigantea' has whitish flowers. The biggest physical difference between the two species that can be distinguished is the colour of their blooms while they are in bud or bloomed state [203].

The Calotropis procera (Apocynaceae and Asclepiadoideae), also known as "milkweed or aak" in India, as Madar and Ushar in Greek-Arabian traditional medicine, and "Apple of Sodom" native to the Dead Sea and Sodom, Israel, and other arid locations, where extracts and powders of various Calotropis components is widely recognised for its therapeutic qualities and have been utilised since ancient times. It is a xerophytic, erect shrub about 3 to 6 ft (0.91 to 1.83 m) tall that grows across the tropics of Africa and Asia. It is abundantly grown in barren and semi-barren locations without the use of irrigation, artificial fertilisers, pesticides, or other agronomic techniques. Calotropis procera was used medicinally by the ancient Egyptians [204], possibly as far back as the Neolithic period [205], and the plant can also be found in Sudanese traditional medicine [206], as well as in the traditional medicine of North and Central Africa, the Middle East in general, and Central Asia (especially in India). Plant is a good bio-indicator for monitoring pollution levels in urban and suburban region [207]. For centuries, the shrub Calotropis procera has been known as a very promising source of ascaricidal [208], schizonticidal, nematocidal [209], anti-microbial [210], antihelmintic, molluscicidal [211], anticancer [212], cytotoxic chemicals, and many other beneficial properties that make this plant a golden gift for humankind [213]. The plant's leave biomass has the potential to be an excellent adsorbent for the removal of crystal violet from aqueous solutions and it is also utilised in the textile industry [214].

a) Phytochemistry of Calotropis procera

The Calotropis procera (CP) plants contain the cardenolide, proceragenin, while the root bark contains benzoylinesolone and benzoylisolinelone. The leave and stalk contain

calotropin, and calotropagenin while the flower contains calotropenyl acetate, and multiflavenol and the latex contains uzarigenin, and terpenol ester [215].

Rajani et al. [216], describe that, chemical analysis of this plant revealed the presence of triterpenoids, calotropursenyl acetate and calopfriedelenyl, a norditerpenyl ester, calotropternyl ester, oleanene triterpenes such as calotropoleanyl ester, procerleanol A and B, and cardiac glycosides such as calotropogenin, calotropin, uscharin, calotoxin, and calactin. They also studied the presence of cardenolides and anthocyanins in the plant, and also discovered two novel phytoconstituents in the roots of Calotropis procera Linn, i.e. Procerursenyl acetate and proceranol, join the known chemicals N-dotriacont-6-ene, glyceryl mono-oleolyl-2-phosphate, methyl myrisate, methyl behenate, and glyceryl-1, 2-dicapriate-3-phosphate. On the basis of spectrum data analysis and chemical reactions, the novel compounds' structures have been identified as urs-18 alpha-II-12, 20 (30)-diene-3 beta-yl acetate, and n-triacontan-10 beta-ol. -amyrin, -amyrin, lupeol, -sitosterol, and flavanols like quercetin-3-rutinoside have also been discovered in the root bark.

Perwez et al. [217], found Mudarine is the main active ingredient in the leave, along with a bitter yellow acid, resin, and three poisonous glycosides called calotropin, uscharin, and calotoxin. The latex contains a potent bacteriolytic enzyme as well as a highly poisonous glycoside known as calactin (as a defence mechanism, its concentration rises after an insect or grasshopper attack), calotropin D I, calotropin D II, calotropin-F I, calotropin F II and a non-toxic protealytic enzyme calotropin (2% - 3%). They made the observation that; calotropin has a higher proteolytic activity than papain, bromelain, as well as coagulates milk and digests meat, gelatin, and casein. Alpha and beta-amyrin, beta-amyrin, teraxasterol, gigantin, giganteol, isogiganteol, beta-sitosterol, and a wax are all found in the complete plant.

b) Medicinal uses of Calotropis procera

The following biological activities of the plant have garnered a lot of attention: C. procera has been shown to have anticancer, antifungal, and insecticidal action in prior pharmacological investigations [218]. The plant's blooms have hepatoprotective, anti-inflammatory, antipyretic, analgesic, and antibacterial properties, as well as larvicidal action. The plant's latex is said to have analgesic, wound-healing, anti-inflammatory, and antibacterial properties, while the roots are said to have antifertility and anti-ulcer properties [219]. Figure 2.14 depicts the various medicinal applications of Calotropis procera.

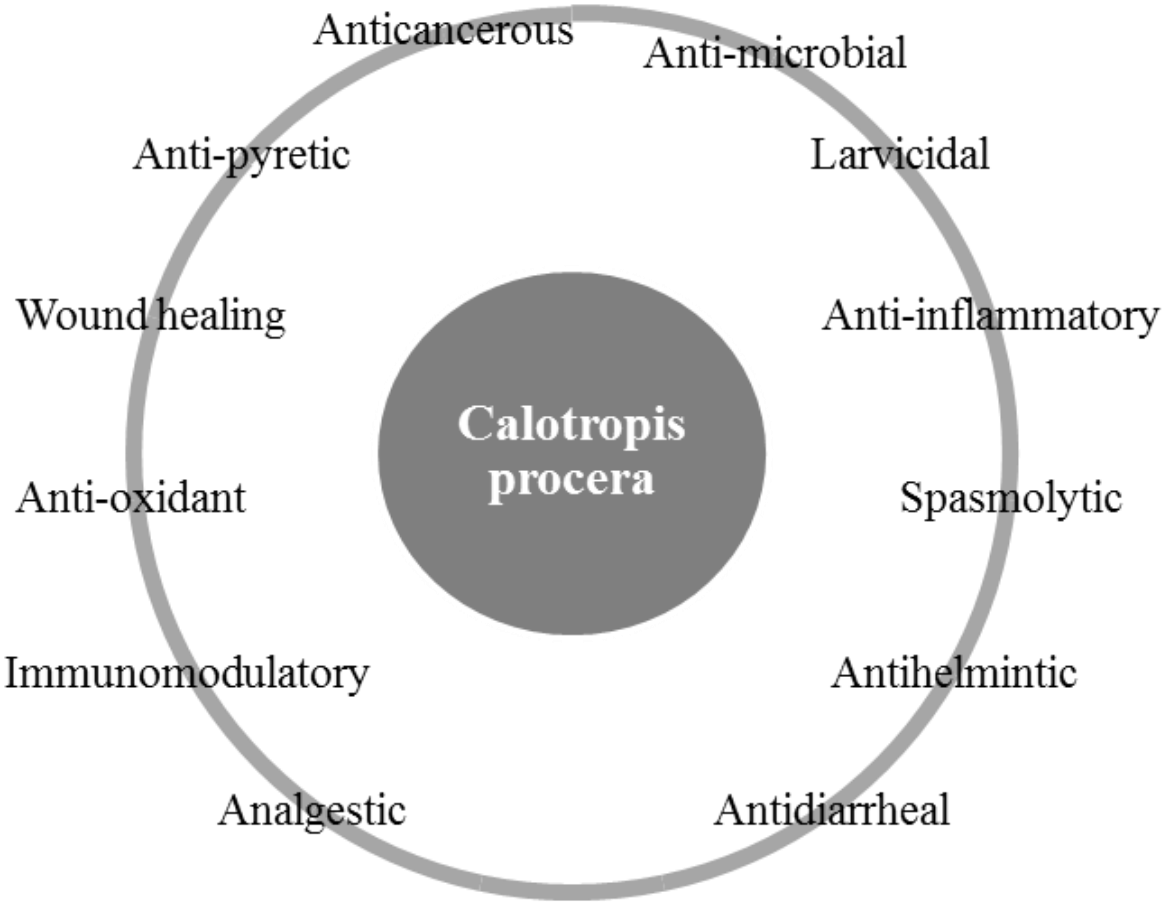


Figure 2.14: Medicinal applications of Calotropis procera

c) Synthesis of Nanoparticles using Calotropis (Milkweed)

Chaudhuri et al. [122], used Calotropis leave extract with zinc acetate salt in the presence of 2 M NaOH to make zinc oxide nanoparticles. The combination of 200 mM zinc acetate salt and 15 ml leave extract proved suitable for producing extremely monodisperse crystalline spherical nanoparticles with a diameter of less-than 20 nm. In open-air trenches, they investigated the impact of biogenic zinc oxide (ZnO) nanoparticles on the growth and development of tree seedlings in the nursery stage. The UV–Vis absorption maximum peaks at around 350 nm, which is typical of ZnO nanoparticles. The solitary peak is at 11 nm (100 percent) and the Polydispersity Index is 0.245, according to DLS data. These were extremely crystalline ZnO nanoparticles with an average size of 10 nm, according to XRD measurements.

Gawade et al. [220], used aqueous leave extract of Calotropis procera as a reducing and stabilising agent to establish a new and economical green-way for the synthesis of ZnO nanoparticles via biogenic technique. An XRD pattern revealed the hexagonal wurtzite structure of ZnO nanoparticles. The ground excitonic peak of ZnO nanoparticles is represented as an absorption edge at 397 nm in the DRS absorption spectrum. The presence of hydroxyl groups, aldehydes, amines, ketones, and carboxylic acids in the FT-IR spectra indicates that biological reactions are taking place. The particles of ZnO have a spherical form, with sizes ranging from 15 to 25 nm, as seen by TEM pictures. Under UV light, ZnO nanoparticles were used as photocatalysts for the breakdown of methyl orange.

Ayodhya et al. [221], synthesised cadmium sulphide nanoparticles (CdS NPs) using aqueous extract of leave of Calotropis gigantea. To make spherical CdS NPs, the extract was combined with 40 mL of 1mM cadmium acetate and 40 mL of 1mM sodium sulphide. The shape, stability, and particle size of CdS NPs were subsequently determined, and photocatalytic activity was investigated using MB and EY dyes under solar irradiation. Three notable peaks in the XRD pattern of CDs NPs were found at 26.4°, 43.4°, and 51.6°. CdS NPs were discovered to have an average particle size of 12 nm. The stability of CdS NPs and the reduction of both dyes were attributed to electron-donating functional groups of phytochemicals found in an aqueous extract of C. gigantea leave.

d) Synthesis of Silver Nanoparticles (AgNPs) using Calotropis (Milkweed)

According to Sivakumar et al. [176], Calotropis leave extract successfully synthesises yeast-like silver nanoparticles. The nanoparticles were formed at 1:4 ratio of Calotropis leave extract to Silver nitrate solution. The nanoparticles were found to be 83.7nm, 15.9nm, and 11.8nm in size, as determined by XRD data. In UV spectra, the nanoparticles revealed a distinctive absorption peak at 420 nm. FTIR research suggested the possibility of protein as a stabilising factor responsible for the production of silver nanoparticles.

Sagadevan et al. [177], used a 1:10 ratio of Calotropis procera root extract to synthesis AgNPs in an aqueous solution of 1mM $AgNO_3$. The colour change and UV–Vis spectra were used to track the synthesis and stability of the reaction mixture. Within 10 minutes of adding the extract, the colour of the colloid changed from yellowish-green to golden yellow, then yellowish-brown. The spectrum study concentrated on the wavelength range 400–460nm, with the absorbance maxima (kmax) at 455nm due to the usual SPR property of silver (Ag^0) at 100 ^oC. Using the Debye–Scherrer equation, they computed the average crystallite size of AgNPs, which was found to be roughly 22nm at 100 ^oC.

Ali et al. [222], biosynthesized AgNPs by using the aqueous leave extract of Calotropis gigantean. They tested the bioactive components of C. gigantea for their capacity to function as biocatalysts in the reduction of Ag^+ to Ag^0. An absorption band peaking at 450 nm was seen in the UV-Visible spectra of biosynthesized AgNPs. The absorption wavelength in the visible area is supported by the colour of the solution. The TEM findings revealed that the biosynthesized AgNPs were spherical in form and ranged in size from 10 to 70 nm. Furthermore, AgNPs were scattered equally and agglomeration was minimal.

Mohamed et al. [223], used the latex of the Calotropis procera to make AgNPs. The production of AgNPs was validated by UV/vis spectroscopic measurements of the coloured solution, which revealed a peak band at 400–430 nm. The crystalline character of the produced AgNPs was verified by the XRD data. AgNPs were found to be spherical and well scattered in TEM images, with sizes ranging from 4 nm to 26 nm.

2.5 TESTING AND ANALYSIS

Textile nano-composites properties are greatly influenced by their composition, particle size, and interfacial contact than other factors. Even tensile, thermal, and other properties of nano-composites are pretty well affected by the interfacial interactions that occur between textile polymer and nanoparticles, which is generally a subject of the preparation technique. Thereby to comprehend the structure completely, it becomes utmost importance to get these measures precisely.

There are diversified methods used by the researchers to analyse the phytochemicals present in the plant extract, chemical structure, microstructure, and morphology of nano-composites, as well as their physical properties. Out of them, some characterization techniques are frequently used to avail detailed structure-property interactions required to understand the scientific behaviour of green nano textile composites [224].

The morphology of nanoparticles and nano-composites can be observed using Visual analysis, Spectroscopic analysis (XRD, UV-Visible spectroscopy, FTIR, DLS, etc.), and Microscopic analysis (SEM, TEM, EDS or EDAX, etc.). Such techniques are the commonly used powerful technique to investigate the chemical structure and characteristics of nanoparticle loaded composites. It's also a usual requirement to specify the mechanical properties of nano-composites from multiple perspectives, such as physical, low-stress, comfort, and so on [225].

2.5.1 PHYTOCHEMICAL ANALYSIS OF PLANTS EXTRACTS

Primary and secondary phytochemicals are natural bioactive substances found in plants and are split into two categories. These chemicals are categorised based on their roles in plant metabolism. Primary compounds include amino acids, carbohydrates, proteins, and chlorophyll, whereas secondary compounds include alkaloids, terpenoids, phenolic compounds, and many others [226]. There are number of non-nutritive phytochemicals that have protective or disease-preventive qualities. These compounds are produced by plants to defend themselves, and they can help protect humans from illness [227].

Volatile oils, Alkaloids, Glycosides, Flavanoids, Tannins and Polyphenolic compounds, Carbohydrates, Proteins, Fixed oils and Fats, Terpenoids [228] are precursors for the synthesis of useful medicines found in medicinal plant parts. The phytochemical analysis

of crude plant extracts is critical in determining their possible pharmacological effects. It is now feasible to do physical examination of crude extracts using separation methods and instrumental analysis, which can be both qualitative and quantitative [229,230].

2.5.1.1 Gas chromatography–Mass spectrometry (GC-MS)

The Gas chromatography–Mass spectrometry (GC-MS) is an analytical technique for identifying distinct chemicals within a test sample that combines the properties of gas chromatography and mass spectrometry. Drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown materials are all examples of GC-MS applications. GC-MS may identify trace components in materials that were previously considered to be unidentifiable. It also enables the identification and analysis of material available in a very small quantity [231].

Operational Principles:

The gas chromatograph and the mass spectrometer are the two main components of the GC-MS (Figure 2.15). The molecular separation qualities of a capillary column in a gas chromatograph are determined by the column's parameters; length, diameter, film thickness as well as the phase properties; e.g., 5 % phenyl polysiloxane. As the sample travels the length of the column, the difference in chemical characteristics between various molecules in a mixture, as well as their relative attraction for the stationary phase of the column, will encourage molecular separation. The molecules are captured by the column and subsequently elute at various intervals referred as the retention time-RT, allowing the mass spectrometer downstream to catch, ionise, accelerate, deflect, and detect the ionised molecules independently [232]. The mass spectrometer does this by ionising each molecule and identifying these pieces based on their mass-to-charge ratio (m/z). Many method standardisation organisations utilise matching algorithms like Probability Based Matching and dot-product matching with methods of analysis created via spectral library searches. National Institute of Standards (NIST), Wiley, American Academy of Forensic Sciences (AAFS), and instrument makers are all recommended places to look for libraries [231].

When these two components are utilised in combination, they can identify substances considerably more precisely than if they were employed alone. By using gas chromatography or mass spectrometry alone, it is impossible to accurately identify a specific chemical. While mass spectrometry requires a very pure sample, traditional gas chromatography detectors;

such as the Flame ionisation detector, cannot distinguish between multiple molecules that take the same amount of time to travel through the column; i.e., have the same retention time, resulting in two or more molecules co-eluting [232]. In a mass spectrometer, two distinct molecules might sometimes exhibit a similar pattern of ionised fragments (mass spectrum). Combining the two techniques decreases the risk of mistake since two separate molecules are unlikely to react similarly in both a gas chromatograph and a mass spectrometer. As a result, when an identifying mass spectrum emerges at a certain retention period in a GC-MS study, it improves the likelihood analysis of interest present in the sample [231].

Ionization Techniques: After the molecules have travelled the length of the column, passed through the transfer line, and entered the mass spectrometer, they are ionised using a variety of ways e.g. electron impact (EI) or chemical ionization (CI), with normally just one method being employed at a time. After the sample has been fragmented, it is generally detected using an electron multiplier, which converts the ionised mass fragment into an electrical signal that can be detected.

The analysts entering the source in GC/MS are ionized by electron impact (EI) or chemical ionization (CI) and the mass analyzer is usually a single quadrupole.

Electron Ionization (EI): Previously known as electron impact ionisation and electron bombardment ionisation, is an ionisation process in which energetic electrons contact atoms or molecules in the solid or gas phase to create ions. EI was one of the earliest mass spectrometry ionisation procedures to be developed. However, this type of ionisation is still widely used. Because it employs extremely energy electrons to create ions, this approach is classified as a hard (high fragmentation) ionisation method. This results in a lot of fragmentation, which can help in figuring out the structure of unknown substances. For organic molecules with molecular weights less-than 600, EI is the most helpful. When combined with other separation procedures, this technology can also identify a variety of additional thermally stable and volatile chemicals in solid, liquid, and gas phases.

Chemical Ionization (CI): In mass spectrometry, CI is a gentle ionisation method. Burnaby Munson and Frank H. Field were the first to introduce this in 1966. This method belongs to the field of gaseous ion-molecule chemistry. Electron ionisation is used to ionise reagent gas molecules, which then react with analyse molecules in the

gas phase to produce ionisation. Some typical versions of this approach include negative chemical ionisation (NCI), charge-exchange chemical ionisation, and atmospheric-pressure chemical ionisation (APCI), as well as atmospheric pressure photoionization (APPI). Identification, structural elucidation, and quantification of organic molecules are all essential uses of CI. Chemical ionization's utility is not only limited to analytical chemistry; it also has applications in biochemical, biological, and medical domains.

***Procedure for GC/MS Analysis*:**

1) Inject sample into Gas Chromatograph (GC) - The sample is injected into a port that is heated to 300°C, at which point the material gets volatilized.

2) Separation of gaseous components as they pass through the column – The column is wrapped in a special oven with temperatures ranging from -20° to 320°. The surface of the column is covered with a substance that separates the chemical components in the sample by size and/or polarity. Sample components that are more volatile and smaller in size will pass through the column faster.

3) Analysis using a mass spectrometer (MS) – The divided components leaves the column directly into the MS, as per three internal steps:

a. Ionization source: - electrons are blasted into components, forcing them to break apart and form positively charged ions.

b. Filter: - Ions flow through an electromagnetic field and are filtered according to their mass. As masses travel through the ionisation source, analysts set a specified range of masses to be permitted through.

c. Detector: - after counting the number of filtered ions, the data is delivered to a computer, which generates a mass spectrum, basically a distribution of ions of various sizes.

The mass spectrum is compared to reference libraries of over 275,000 distinct spectra to determine the components. Analysts create a standard curve with known amounts of each substance to quantify chemicals within the studied sample.

Important uses of GC-MS:

- Identifying and calculating the amount of volatile organic chemicals in a combination
- Recognizing and measuring unidentified samples
- De-formulation of chemicals and chemical products
- Experiments on outgassing
- Characterisation of antibacterial coatings/finishing
- Detecting the presence of residual solvents
- Investigation of oils and petrochemicals
- Detecting minute amounts of contaminants in liquids or gases
- Plastic extracts are being evaluated
- Unknown pollutants must be identified

Strengths of GC-MS:

- Separation of complicated mixtures allows the identification of organic components.
- Quantitative research
- Organic contamination trace determination (low to mid-ppb level for liquid matrices, low nanogram level for solid matrices — Dynamic Headspace Analysis)

Limitations of GC-MS:

- Initially, target molecules must be either volatile or derivatized
- Secondly, non-volatile matrices (wafers, oils, metal pieces, and so forth) need further preparation (extraction, outgassing, etc.)
- Finally, atmospheric gases (CO_2, N_2, O_2, Ar, CO, etc.) pose a problem

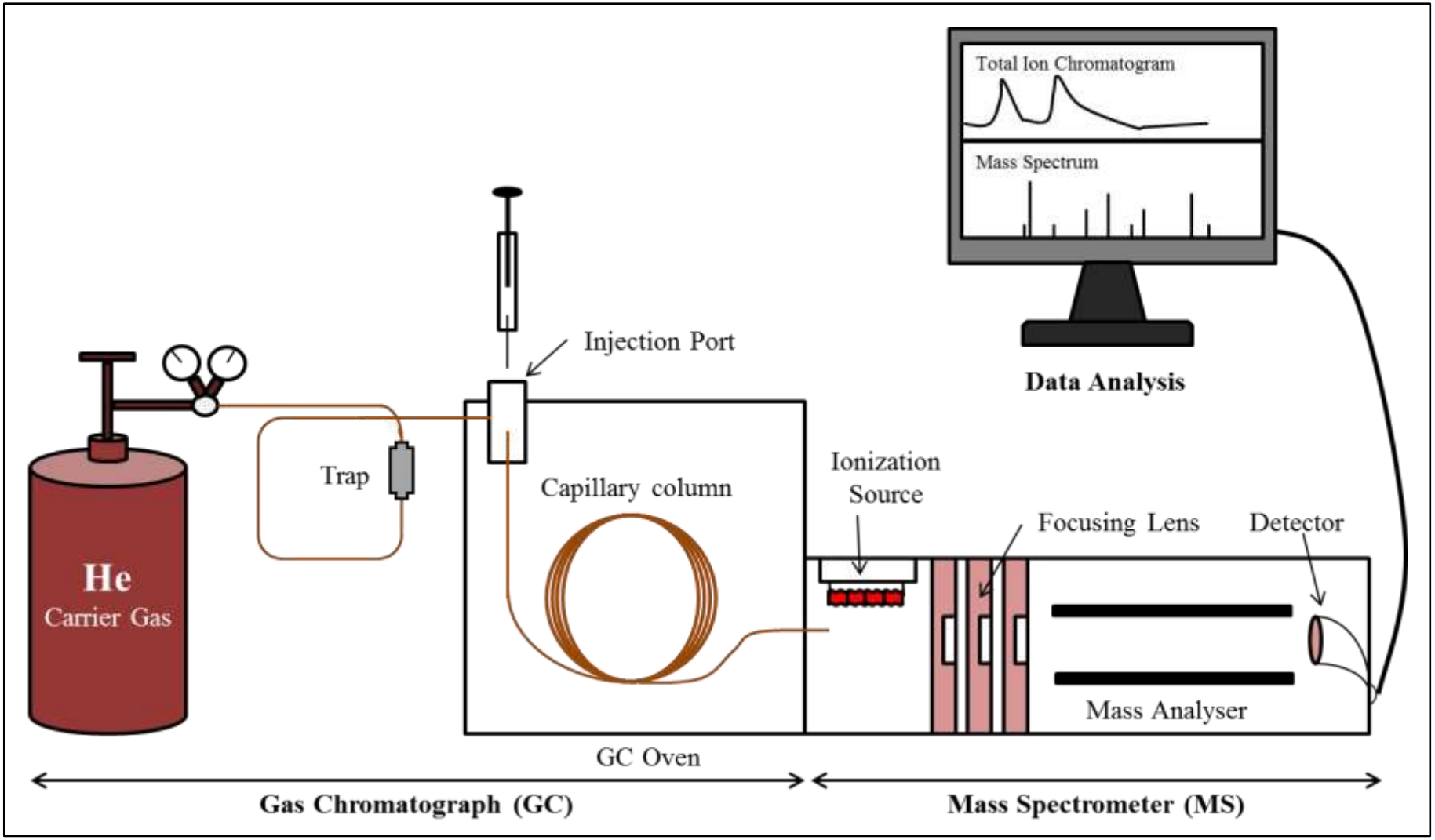


Figure 2.15: Gas chromatograph and mass spectrometer

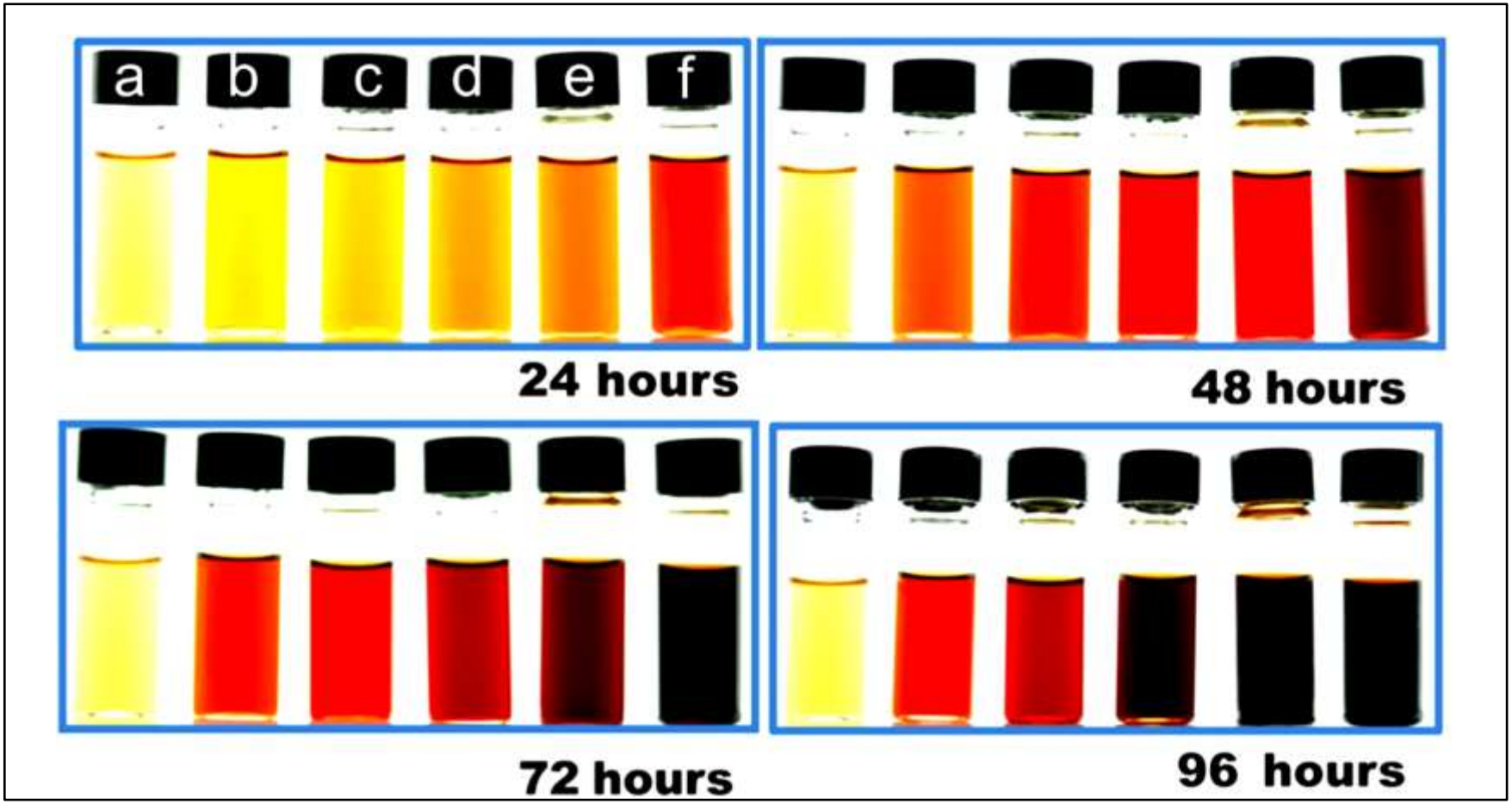


Figure 2.16: Visual appearance of vials containing the Rumex hymenosepalus extract and $AgNO_3$ solution after different reaction times [237]

The vials correspond to different $AgNO_3$ concentrations: (a) pure extract, (b) 2.5 mM, (c) 5 mM, (d) 7.5 mM, (e) 10 mM, and (f) 15 mM. The change in colour is an indication of the growth of silver nanoparticles.

2.5.2 CHARACTERISATION TECHNIQUES FOR NANOPARTICLES FORMATION

Imaging nanostructured materials and characterise their physical and chemical characteristics using a variety of approaches. Apart from visual analysis, there are mainly two types of characterization methods in general; imaging by spectroscopy and microscopy analysis. These methodologies used were created expressly to fulfil the demands of nanomaterial characterization.

1. Visual analysis of Nanoparticles

2. Spectroscopic analysis of Nanoparticles

3. Microscopic analysis of Nanoparticles

2.5.2.1 Visual analysis of Nanoparticles

The visual colour change perceives elementary information about the formation of nanoparticles. The colour of the solution changes from bright to dark on the development of nanoparticles, endorsing visual observation about the existence of nanoparticles. This colour change is usually initiated by the nanoparticles' collective oscillations of free conduction electrons [233,234]. Colour fluctuation is also caused by the changes took place in nanoparticle surface plasmon resonance (SPR) during production [235]. The depth of the colour in the solution increases with the concentration of the colloidal. This is attributed to the factual derived that as the concentration of colloidal nanoparticles rises, produced ions aggregate, resulting in the creation of bigger nanoparticles [236]. In other words, the shift in colour from light to dark has been caused by the size change of nanoparticles from nano to micro/macro structures.

Augustine et al. [235], discover primary information about the synthesis of silver nanoparticles by observing the visual colour change of the colloidal solution. Accordingly, the colour of the solution changes from white to pale yellow to brick red as silver nanoparticles formed, indicating the presence of silver nanoparticles in the colloidal. A very light-yellow colour was realized at a 1 mM silver nitrate concentration. The redness of the solution was increased with the concentration of silver nitrate and this behaviour was continued up to 5 mM. This has happened since as the concentration of silver nitrate rises in

the solution, formed silver ions clump together and resulting in the formation of larger silver nanoparticles.

Rodríguez-León et al. [237], noted that the samples altered their visual appearance quickly after the addition of the plant extract for all selected $AgNO_3$ concentrations, ensuring the occurrence of a reduction process. The reacting combination was beginning with a somewhat yellowish liquid, which progressively become orange, red, and then brown. They illustrated vials containing reacting materials at various $AgNO_3$ concentrations (0, 2.5, 5, 7.5, 10, and 15 mM) and reaction periods after the beginning; 24, 48, 72, and 96 hours, the evident temporal evolution is a signature of silver nanoparticle development (Figure 2.16). The shift in hue observed was caused by the high absorption of visible light caused by surface plasmon excitation in nanoparticles.

2.5.2.2 Spectroscopic analysis of Nanoparticles

It represents the study and measurement of spectra created when matter interacts with or emits electromagnetic radiation also referred as spectroscopy. Different spectra are measured depending on the wavelength of the electromagnetic utilised and the sort of contact with matter that happens, from which a lot of information may be gleaned.

I) X-Ray Diffraction (XRD) methods

The X-ray scattering phenomenon is used to reveal the crystal structure of crystalline/semi-crystalline materials. X-rays are scattered by a periodic array of atoms, resulting in defined diffraction patterns that provide a qualitative depiction of atomic groupings inside the crystal lattice. Various methods use X-rays: X-ray fluorescence (XRF), X-ray diffraction (XRD), etc. in the context of nanomaterials, the most important methods are small and wide-angle X-ray scattering (SAXS and WAXS) analysis. These X-Ray Diffraction (XRD) techniques are one such characterisation technologies that have the benefit of simultaneous characterising both the precursor and end products, as well as providing a complete qualitative description of their microstructural behaviour. In contrast to the single-crystal technique, which requires the sample in the form of individual/single/independent crystals, they are the most popular and practical approaches for characterisation of the polymer-based nano-composite. XRD is also a flexible, non-destructive characterisation technique that provides a comprehensive output of chemical composition, and as a result, the crystalline structure of materials. This technique can also be used to determine the size of

nanoparticles [238]. Figure 2.17 represents the basic principle of the X-ray diffraction technique.

Benefits and Applications of XRD:

The XRD technique is a non-destructive method used for:

- Determining structural properties
- Identifying crystalline phases and orientation
    - Parameters of the lattice, Grain size, Strain, Phase composition, Epitaxy, Preferred position, etc.
- Determining the thickness of thin films and multilayers
- Determining the atomic structure

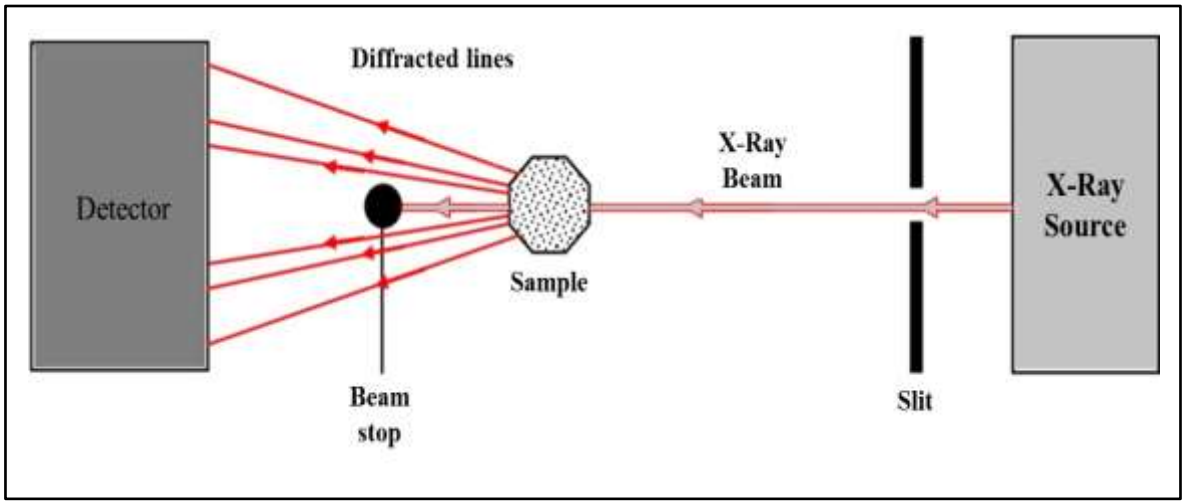


Figure 2.17: Basic principle of X-ray diffraction [239]

II) UV- Visible Spectroscopy

UV spectroscopy, also known as UV–visible spectrophotometry (UV–Vis or UV/Vis), is the study of absorption and reflectance spectroscopy in the ultraviolet and nearby visible parts of the electromagnetic spectrum. The visible range absorption or reflectance has a direct impact on the apparent colour of the substances involved. Atoms and molecules undergo electronic transitions in this area of the spectrum. Absorption spectroscopy measures transitions from the ground state to the excited state and is similar to fluorescence spectroscopy. The fluorescence technique measures transitions from the excited state to the ground state. Figures 2.18 illustrate a simplified schematic of UV-Vis spectrophotometer configurations.

Strengths and limitations of UV-Vis spectroscopy:

Strengths: There are a few key advantages of UV-vis spectroscopy as listed below.

- Because the procedure is non-destructive, the sample can be reused or subjected to additional processing or analysis
- Instruments are simple to use and require little user training prior to use
- Measurements can be made fast, making them straightforward to include in experimental techniques
- Data analysis often necessitates minimum processing, implying that little user training is necessary
- The instrument is relatively affordable to purchase and run, making it suitable for a wide range of laboratories [241].

Limitations: Although the advantages of this strategy appear to be overwhelming, there are several drawbacks:

- Light scattering in liquid samples is frequently produced by suspended particles, which can lead to substantial measurement mistakes
- Ambient light or a loosely fitting compartment in the instrument might potentially cause stray light
- Interference from a number of different absorbing species

- When it comes to creating outcomes, geometrical factors are crucial. For example, misalignment of any of the instrumental components, particularly the cuvette that holds the sample, might result in irreproducible and erroneous findings [241].

Applications of UV-Vis spectroscopy:

- Characterization of extremely tiny nanoparticles
- Analyzing particular structural protein alterations
- Determine the quantities of haemoglobin
- Determining the composition of the battery
- Calculation of the colour index
- Analysis of DNA and RNA
- Pharmacological research
- Treatment of waste-water
- Bacterial culturing
- Analysis of beverages, etc. [242]

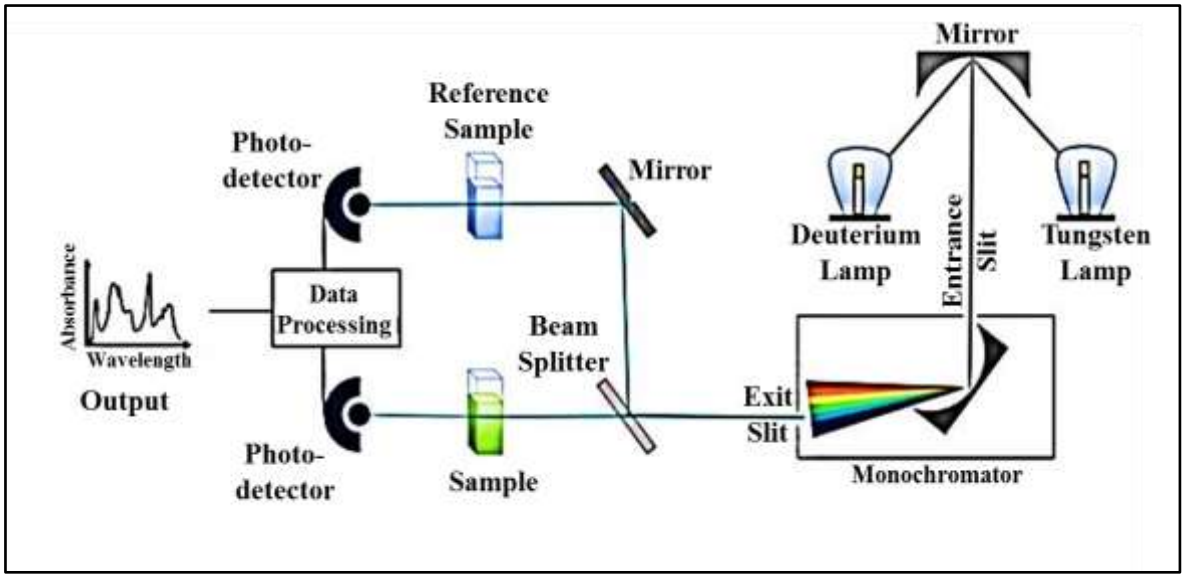


Figure 2.18: Schematic of a UV-Vis spectrophotometer's key components [240]

III) Fourier Transform Infrared Spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (FTIR) is a technique for obtaining an infrared spectrum of a solid, liquid, or gas's absorption or emission. An FTIR spectrometer obtains high-resolution spectral data over a large spectral range at the same time. This gives it a big advantage over a dispersive spectrometer, which only measures intensity across a small range of wavelengths at a time. The phrase "Fourier-transform infrared spectroscopy" comes from the fact that raw data must be converted into a spectrum using a Fourier transform; a mathematical technique. The basic operating principle of the FTIR technology is depicted in Figure 2.19.

Applications of the FTIR:

FTIR can be used invariably for the application where a dispersive spectrometer has been used previously. Furthermore, its increased sensitivity and speed have opened up new areas of use. Spectra can be measured even though a little amount of energy enters the detector, and scan rates can exceed 50 spectra per second. Fourier transform infrared spectroscopy is widely employed in study domains such as geology, chemistry, materials, and biology. The important application areas for the FTIR includes [243];

- Nanomaterials and biological materials
- Imaging and microscopy applications
- Nanoscale and diffraction-limited spectroscopy
- Use as a Detector, in chromatography.
- Thermo-gravimetric analysis-infrared spectrometry (TG-IR)
- Determination of water content in polymers and composites, etc.

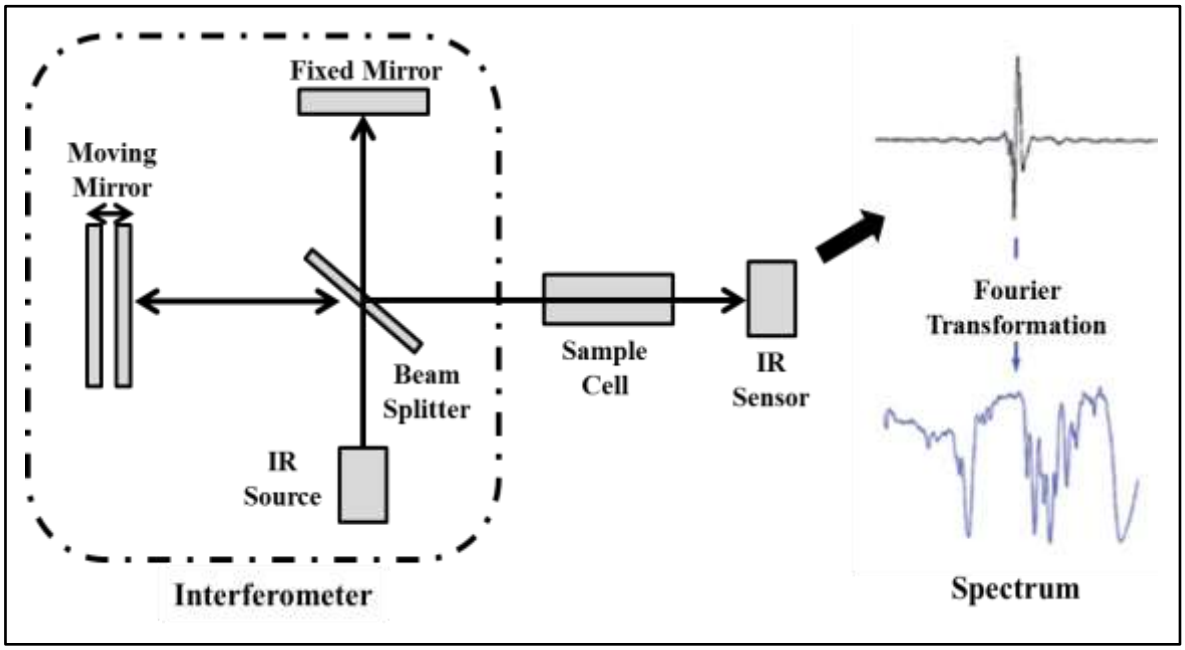


Figure 2.19: Represents the basic principle of the FTIR technique

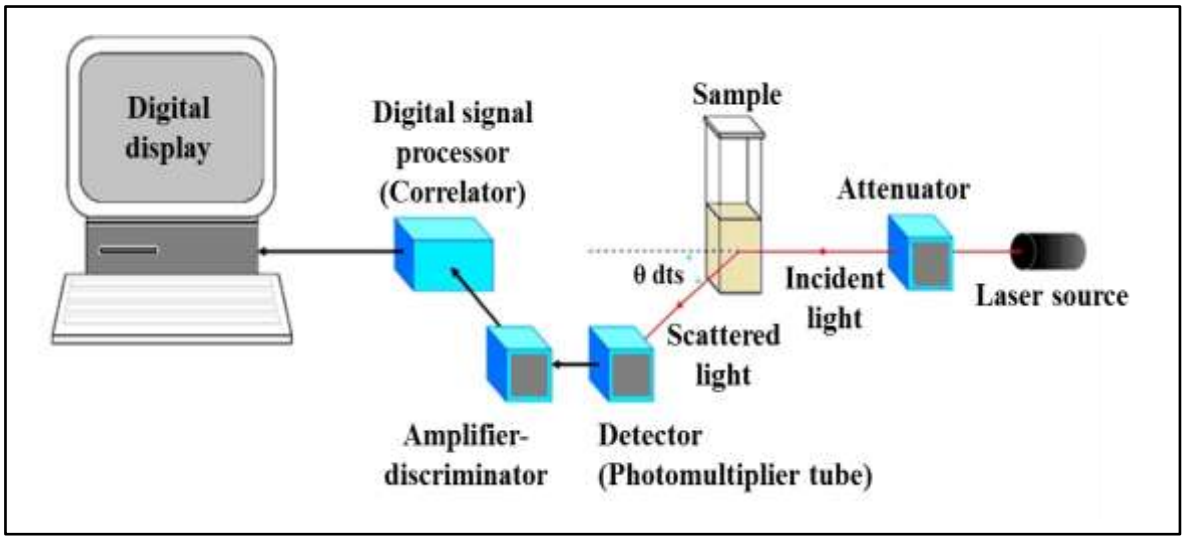


Figure 2.20: Core premise of the Dynamic Light Scattering (DLS) [245]

IV) Particle size Analysis by Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) is the most widely used method for determining particle size distribution profile in the nanoscale range, suspended in solution or polymers in solution. The intensity or photon auto-correlation function is commonly used in DLS to examine temporal variations [also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QELS)]. The autocorrelation function (ACF) in time domain analysis normally decays from zero delay time, and quicker dynamics owing to smaller particles result in faster de-correlation of dispersed intensity trace. The intensity ACF has been demonstrated to be the Fourier transform of the power spectrum, implying that DLS measurements can be done equally in an effective way in the spectral domain. DLS may be used to investigate the behaviour of complicated fluids such as concentrated polymer solutions [244]. The dynamic light scattering (DLS) technique's core premise is depicted in Figure 2.20.

Applications of DLS:

Compared to other approaches, dynamic light scattering provides a number of advantages. Experiments using a wide variety of sample buffers and temperatures, as well as concentrations, can be carried out. DLS is also a non-invasive approach that needs a little bit of material and quickly offers accurate estimations of preparation quality. Some examples of DLS applications used to analyse a wide range of materials/samples are as follows [246]:

- Measurement of sizes of nanoparticles
    - Such as nano gold, protein, latex, colloid, etc.
- Studies of protein-protein interactions
- Detection of protein aggregation
- Homogeneity of milk samples or evaluation of homogenization efficiency

2.5.2.3 Microscopic analysis of Nanoparticles

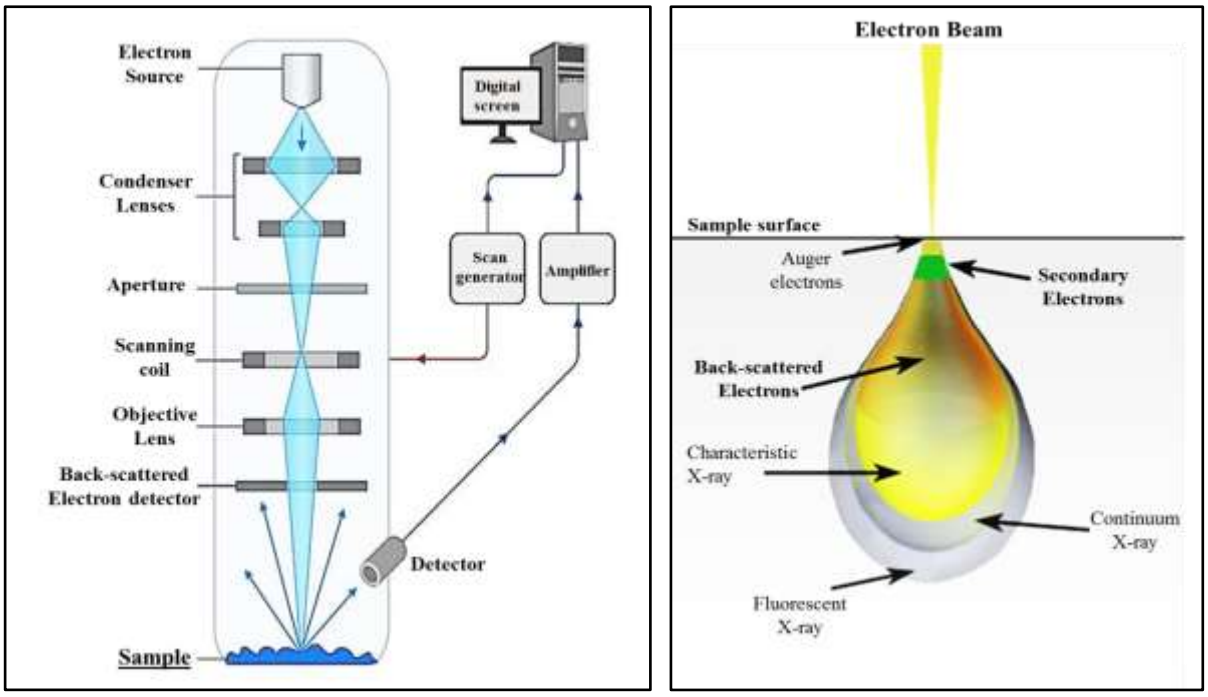
An optical microscope magnifies images of small samples by using visible light and a lens system, thereby also known as a light microscope. Optical microscopes are the most basic and oldest type of microscope. But, they have limited resolving power near about 200 nm. A single object smaller than these limitations is not distinguishable and is referred to as a 'fuzzy object.' This is now known as the visible light diffraction limit.

To address the limits imposed by the diffraction limit of visible light, microscopes using electron beams illumination of the material have been developed. These electron microscopes possess a far higher resolving power than light-based microscopes that employ electromagnetic radiation and are capable of achieving magnifications up to 2000 times. Hence, the wavelength of the radiation used by both electron and light microscopes imposes resolution restrictions. The electron microscope has higher resolution and magnification because an electron's wavelength is substantially shorter than that of a photon of visible light [247].

SEM (scanning electron microscope) and TEM (transmission electron microscope) are two popular examples of such electron microscopes. These microscopes are conceptually similar to optical microscopes in that they both use radiation to visualise a sample: photons in the case of an optical microscope and electrons (particles) in the case of electron microscopes [247].

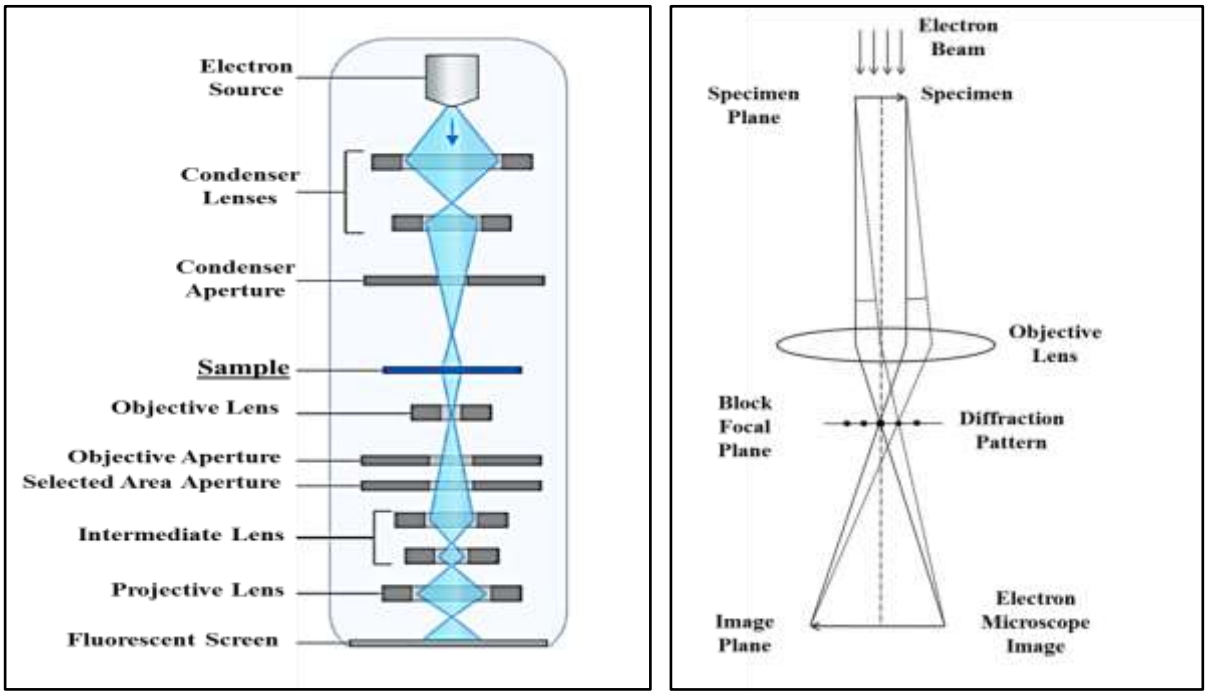
I) Scanning Electron Microscopy (SEM)

A concentrated beam of high-energy electrons is employed in scanning electron microscopy (SEM) to generate a range of signals at the surface of solid specimens. The signals produced by electron-sample interactions provide information about the sample such as its exterior morphology (texture), chemical composition, and the crystalline structure and orientation of the materials that comprise the sample. In most applications, data are collected across a certain area of the sample's surface, and a 2-dimensional image displaying spatial changes in these properties is generated. Using traditional SEM techniques, areas spanning in width from about 1 cm to 5 microns can be scanned in scanning mode (magnification ranging from 20X to approximately 30,000X, spatial resolution of 50 to 100 nm). The SEM can also perform analyses on specific point locations on the material; this method is particularly effective in determining chemical compositions (through Energy Dispersive X-ray Spectroscopy, EDS), crystalline structure, and crystal orientations (using Electron backscatter diffraction, EBSD). The basic principle of scanning electron microscopy (SEM) and Schematic of electron beam interaction are depicted in Figure 2.21(a) and (b) respectively.



(a) **(b)**

Figure 2.21: a) Basic principle of the Scanning electron microscopy (SEM), and b) Schematic of electron beam interaction



(a) **(b)**

Figure 2.22: a) Basic principle of the Transmission electron microscopy (TEM), and b) Schematic of electron beam diffraction

Applications of the SEM:

It's used in a wide range of sectors, including industrial applications, nanoscience research, bio-medical research, and microbiology.

- Used in energy-dispersive X-ray spectroscopy, e.g. for spot chemical analysis.
- Identify the morphological structure of fibre, yarn, or fabric.
- Used to examine cosmetic components that are extremely small in size.
- Microorganism filament architectures are studied using this technique.
- Used to investigate the geography of industrial elements.

II) Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is a type of microscopy that visualises objects and generates a highly magnified image using a particle stream of electrons. A suspension on a grid or an ultrathin segment less-than 100 nm thick is the most common specimen. The interaction of the electrons with the sample as the beam passes through the specimen creates a picture. The picture is then enlarged and focussed onto an imaging device, such as a fluorescent screen, a layer of photographic film, or a sensor linked to a charge-coupled device, such as a scintillator. TEM has the ability to magnify items by up to 2 million times.

In the physical, chemical, and biological sciences, transmission electron microscopy is a common analytical technique. TEM has also occupied an important position in biological and medical sectors, including; cancer research, virology, and materials science, as well as pollution, nanotechnology, and semiconductor research, similarly in palaeontology and palynology. The basic principle of transmission electron microscopy (TEM) and Schematic of electron beam diffraction are depicted in Figure 2.22(a) and (b) respectively.

Applications of TEM:

TEM is employed in a multitude of domains, including biology, microbiology, nanotechnology, forensics, and more. Some of the most valuable applications are:

- Visualised and studied bacteria, viruses, and fungal cell structures.
- To investigate and distinguish between plant and animal cells.
- To analyse the many forms and sizes of microbial cell organelles.

- To analyse bacterium flagella and plasmids.
- It's also used to study nanoparticles like ZnO, Ag, Au nanoparticles in nanotechnology.
- It is utilised to detect and identify fractures and damaged microparticles, as well as to enable particle repair mechanisms.

III) Energy Dispersive X-ray Spectroscopy (EDS or EDAX)

Energy-dispersive X-ray spectroscopy (EDS, EDAX, EDXS, or XEDS) also known as energy dispersive X-ray analysis (EDXA or EDAX) or energy dispersive X-ray microanalysis (EDXMA), is a technique for determining the elemental composition or chemical characterisation of a sample.

EDS systems are typically used in conjunction with an electron microscope, such as a scanning electron microscope (SEM) or a transmission electron microscope (TEM). The EDS method is based on the emission of a specimen's unique X-rays. The studied sample is bombarded with a beam of high-energy charged particles (electrons or protons). When an electron from a higher binding energy electron level falls into the core hole, it emits an X-ray with the energy of the difference between the binding energies of the electron levels. The peaks connected to the elemental makeup of the studied sample are displayed in an EDS spectrum. This characterisation method can also be used to create a sample's elemental mapping. The basic principle of Energy-dispersive X-ray spectroscopy (EDS) is depicted in (Figure 2.23).

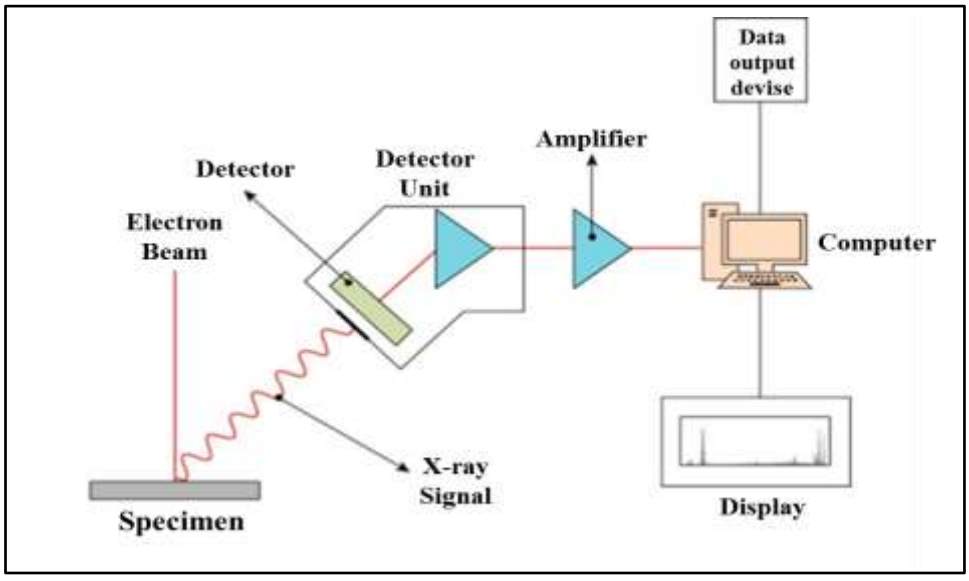


Figure 2.23: Schematic of the EDS spectroscopy technique

Applications of EDS's include:

- Evaluation and identification of materials
- Identification of contamination
- Profiles of elemental diffusion
- Phosphorus content in glassivation
- Failure study
- Voluminous spots analysis ranging in size from 1 micron to 10 cm in diameter
- Identification of Unknowns
- Location and identification of stringers
- Screening for quality assurance
- Verification of the materials
- Specification and certification for plating

2.5.2.4 Size distribution of nanoparticles by the Digital Image Processing Program/Software

Digital image processing becomes a more widely used technique in nano-structural investigation, and it serves well as an excellent tool in determining the morphology and position of self-assembled metallic nanoparticles for high-density recording. Micro- and nanoscale characterizations have relied heavily on images from optical and electron microscopes. Structures' morphology can be analysed, but qualitative examination necessitates time-consuming visual inspections. Such characterizations can now be facilitated due to recent advancements in the field of digital image processing [248]. Digital image processing techniques/programs/software, such as 'ImageJ' Software and Otsu' thresholding are commonly employed for this purpose.

I) 'ImageJ' Software: 'ImageJ' is a Java-based image processing tool created by the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation (LOCI, University of Wisconsin). 'ImageJ' was built with an open architecture that allows for customization through Java plugins and macros. With 'ImageJ's built-in editor and a Java compiler, custom acquisition, analysis, and processing plugins can be created. From three-dimensional live-cell imaging to radiological image processing, multiple imaging system data comparisons, and automated haematology systems, user-written plugins make it feasible to handle a wide range of image processing and analysis challenges. 'ImageJ' is a popular platform for nanoparticles image processing due to its plugin design and built-in development environment. 'ImageJ' also allow you to count and size a large number of nanoparticles in a short interval of time.

II) *Otsu' Thresholding:* Otsu's approach, named after Nobuyuki Otsu, is used to do automatic image thresholding in computer vision and image processing. The method returns a single intensity threshold that divides pixels into two classes: foreground and background, in its most basic form. This limit is set by reducing intra-class intensity variance or, in other words, maximising inter-class variation. Otsu's approach is a discrete one-dimensional version of Fisher's Discriminant Analysis, and connected to Jenks optimization method. It is comparable to a globally optimal k-means done on the intensity histogram.

Wang et al. [249], measured the diameter, nonsphericity, Au-core diameter, Ag-shell thickness, and degree of the off-centered Au-core of the Au/Ag-core/shell NPs using 'ImageJ' software. They used the software's particle analysis technique to determine the NP sizes and nonsphericity. Calculation of Au-core diameter and Ag-shell thickness were done by measuring the distance between eight radially spaced straight lines originating from the centre of the Au-core and the outside border of the Ag-shell. They revealed the size and nonsphericity of the Au/Ag-core/shell NPs in STEM (Scanning transmission electron microscope) images by 'ImageJ' analysis. Each NP's STEM picture is a two-dimensional projection of that specific NP. Individual NPs had a mean diameter of 20.5 ± 2.4 nm and an average nonsphericity of 1.14 ± 0.1, as determined by the major axis to minor axis ratio. Calculations for size and nonsphericity were based on the study of 76 particles. The NPs ranged in size from 13.8 to 24.8 nm, and the majority varied significantly from a perfect sphere (e.g., more than 60% of the particles had a major-axis/minor-axis ratio greater than 1.1).

Phromsuwan et. al. [248], revealed that the image processing approach based on the Canny edge detector and edge linking algorithm can be used to examine TEM images of magnetic nanoparticles with an average diameter of less-than 5 nm. To minimise noise and enhance the image, binary image processing including Otsu's thresholding, closing, and filling stages are also required. Quantitative differentiation is made between two images of nanoparticles with uneven size distributions induced by the reducing agent. The diameter and cross-sectional area of nanoparticles produced by this procedure are bigger than those acquired through one-by-one inspection.

2.5.3 TESTING OF NANO-COMPOSITES MATERIALS

Nano-composites (e.g. woven, nonwovens) have been used in the medical field since the Second World War when there was a need for novel and huge volumes of medicinal products. Nano-composites were rated as the most effective materials for bacterial barriers in various studies. They were also found to be more effective than other materials at reducing airborne pollution [250].

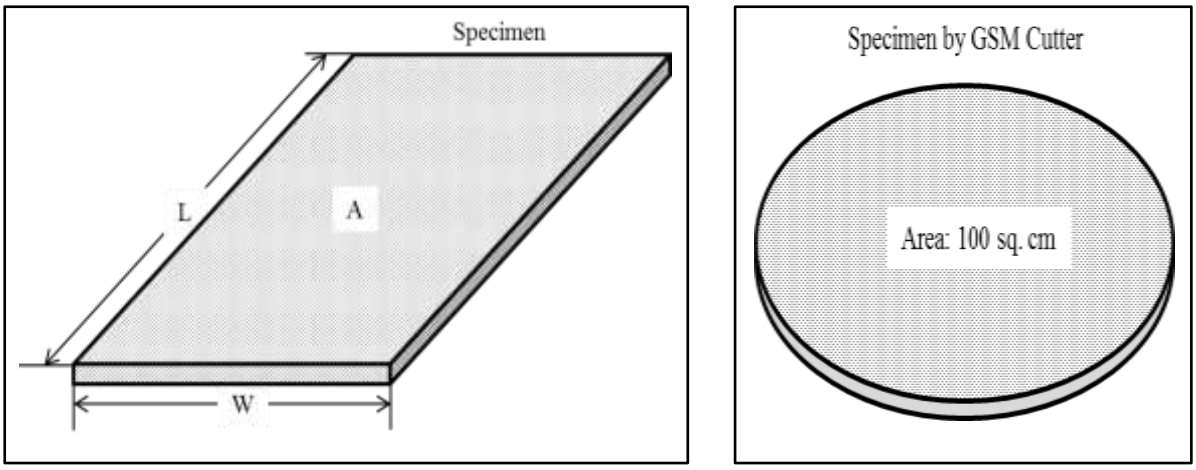
Particularly after the substantial development of nonwovens, their functional nano-composites were designed to fit medical needs and provide performance far superior to their woven counterparts in terms of cost, efficacy, disposability, and so on. Cross-contamination is one of the most common difficulties in hospitals, and it's mostly due to the re-use of woven gowns, masks, and other similar items that become contaminated and spread germs. The introduction of nonwovens aided these problems considerably by the creation of a more cost-effective, disposable alternative.

However, for any form of medical textile material its basic physical properties (e.g., GSM, thickness, etc.), low-stress properties (e.g., stiffness, crease recovery, etc.), comfort-related properties (e.g., air-permeability, moisture management capabilities, etc.), UV protection capacity, antibacterial properties, and toxicological characteristics are mainly accounted in designing product and availing desired performance.

2.5.3.1 Physical assessments

I) Mass per Unit Area (GSM – Gram per square meter)

Mass per unit area (GSM) can be determined by weighing specimen (e.g. woven, nonwoven, composites, etc.) of known dimensions in length (L) and width (W) (e.g. 10cm×10cm) (Figure 2.24) or obtain a consistent specimen size [area (A)]. The dimensions are marked by a cutting device or a template (e.g. GSM cutter). The larger the specimen size, the more accurate the measurement, and most test standards require an area of 10,000 mm^2 or more to be measured. The accuracy of cutting the specimen should be within ±1% of the area.



(a) **(b)**

Figure 2.24: Specimen for GSM calculation a) Rectangular, and b) Circular

Minimum five test specimens should be tested per sample (e.g. fabric). Specimen selection should avoid taking samples from the fabric selvedge (In-case of woven fabric) or close to the ends of a fabric piece. Testing should be conducted in a conditioned atmosphere with preconditioned samples and care should be taken to avoid the loss of fibres/threads during weighing. Results are commonly reported in grams per square metre (g/m^2 or GSM) according to ASTM D 3776-1996, IS1964-2001.

The standards used for the GSM test include:

- ASTM D3776-96(2002) Standard test methods for mass per unit area (weight) of fabric
- ISO 3801-1977 Textiles–Woven fabrics – Determination of mass per unit length and mass per unit area
- AS 2001.2.8-2001 Determination of mass per unit area and mass per unit length of fabrics.

II) Thickness

The thickness of the fabric is one of its essential qualities, providing information about its warmth, weight, or stiffness in use. Thickness measurements are rarely utilised in practice because they are extremely sensitive to the pressure applied during the measurement. Instead, commercially, cloth weight per unit area is utilised more as a thickness indicator.

For a variety of reasons, fabric thickness is a critical metric. Fabric stiffness is dictated by fabric thickness, and hence fabric thickness has a significant impact on how the

fabric drapes and imitates. Fabric thickness is the most essential factor in determining the rate of heat transfer and, as a result, the fabric's 'warmth.' Fabric thickness has an impact on air permeability and moisture absorbency, as well as abrasion resistance.

Thickness is usually measured as the distance between the two fabric surfaces under a certain level of applied pressure, depending on the type of the material, e.g.; woven, nonwovens, etc.. Usually, a thickness gauge is used to measure the thickness of the fabric/textile materials as per ASTM D 1777–96 and IS 7702-1975 standards. For different types of nonwoven textiles, the EDANA (European Disposables and Nonwovens Association)/INDA NWSP (Association of the Nonwoven Fabrics Industry – Nonwovens Standard Procedures) standards, established six standard techniques [251]. Some of them are listed below:

- Nonwoven Fabric Thickness [NWSP 120.1.R0 (15) and ASTM D5729-97],
- High loft Nonwoven Fabric Thickness [NWSP 120.2.R0 (15) and ASTM D5736-95],
- Measuring Compression and Recovery of High loft Nonwoven Fabrics [NWSP 120.3.R0 (15)],
- Determination of Compression and Recovery of High loft Nonwoven Fabrics Method 1: at Room Temperature Using Weights and Plates [NWSP 120.4.R1 (15)],
- Determination of Compression and Recovery of High loft Nonwoven Fabrics Method 1: at increased temperatures [NWSP 120.5.R1 (15)],
- Nonwoven thickness [NWSP 120.6.R0 (15); and BS EN ISO 9073-2:1995].

2.5.3.2 Low-stress properties

Low-stress properties of the nano-composite fabrics have become a quantifiable indication of quality and performance. The end user's/wearer's comfort is intimately linked to the low-stress properties of the textile material, which is significantly altered by uses.

I) Fabric stiffness

The fabric has a unique feature called stiffness. Each fabric has its particular tendency to stand on its own without any support. It is an important aspect in the research of nano-composite fabric used in medical textiles for defining fabric handling and drape; e.g. motion artifact. If all other conditions stay constant, the stiffness of the fabric on bending is highly reliant on its thickness. Thicker the material more will be its stiffness.

Test specimen:

A rectangular strip of nano-composite fabric (6×1 inch) is fixed on a horizontal platform in such a way that it bends downwards and overhang like a cantilever (Figure 2.25).

A number of variables can be calculated using the bending length (l) and angle of bend (θ). The length of the fabric that will bend to a certain extent under its own weight is referred to as bending length (l). It is a measure of stiffness that determines the draping quality of the fabric.

The specimen is usually tested in the length-wise and width-wise direction. Since the relative humidity can affect the results, the test should be conducted at a standard testing atmosphere (Tropical region: Temperature: 27°C ± 2°C and relative humidity: 65 ± 2%). The horizontal platform of the instrument is supported by two side transparent polymer sheet pieces. These side guards have engraved on it the index lines at the standard angle of deflection of 41.5°. The scale is graduated in centimetres (cm) to measure bending length and is also used as the template for cutting the specimens to test size.

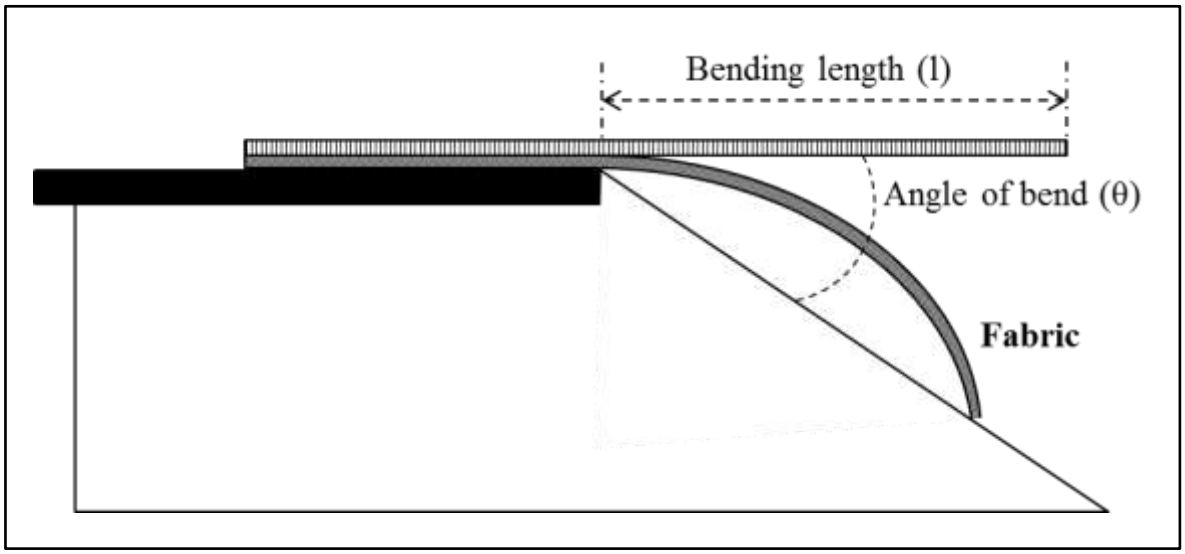


Figure 2.25: Fabric stiffness, cantilever principle

II) Crease recovery angle

It is the ability of a textile material to regain its original shape after being crumpled. Textile material creases or crushes as a result of a complex interaction of tensile, compressive, bending, and torsional forces. Creasing occurs as the fabric is being worn or when a fabric is twisted to the point where a portion of it is stretched beyond its elastic recovery; this variation is not a desirable change in look or comfort. The type of fibre used in the manufacturing of a composite fabric determines its ability to resist creasing in the first place. Some fibres, such as wool and cultivated silk, have good creasing resistance, but cellulosic materials, such as cotton, viscose, and linen, have poor creasing resistance. Fabric crease recovery is measured quantitatively in terms of crease recovery angle.

Instrument:

Shirley crease recovery tester is used popularly to measure the crease recovery angle of the fabric (Figure 2.26). The instrument consists of a circular dial that carries the clamp for holding the specimen. Aligned with the center of the dial, there is a knife edge that acts as an index line during measurement.

Test Specimen:

Test specimens are cut length-wise as well as width-wise using a 2 inches long template. The sample should be free of wrinkled, bowed, or other deformed sections of the fabric, as well as not within 2 inches of the selvedges. Hence, the moisture content of the material affects the results, the tests should be conducted after due conditioning of the fabric in a controlled environment.

2.5.3.3 Comfort-associated properties

Comfort is defined as "the absence of unhappiness or discomfort" or "a neutral condition in comparison to the more active state of joy" [252]. In other words, comfort is the result of the brain's integration of impulses sent up the nerves from a range of peripheral receptors such as smell, smoothness, consistency, and colour. It is a qualitative term that refers to one of the most crucial characteristics of clothes and medical outfits. Some of the important features that have a big impact on the comfort of any garments or medical outfits include air permeability, moisture management capabilities, and so on [253].

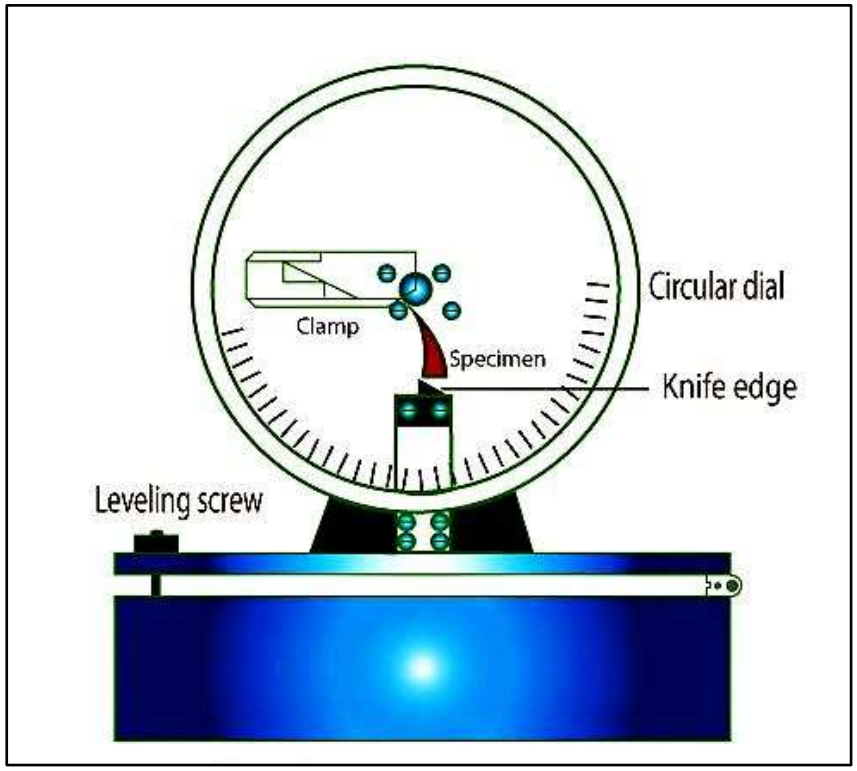


Figure 2.26: Shirley crease recovery tester

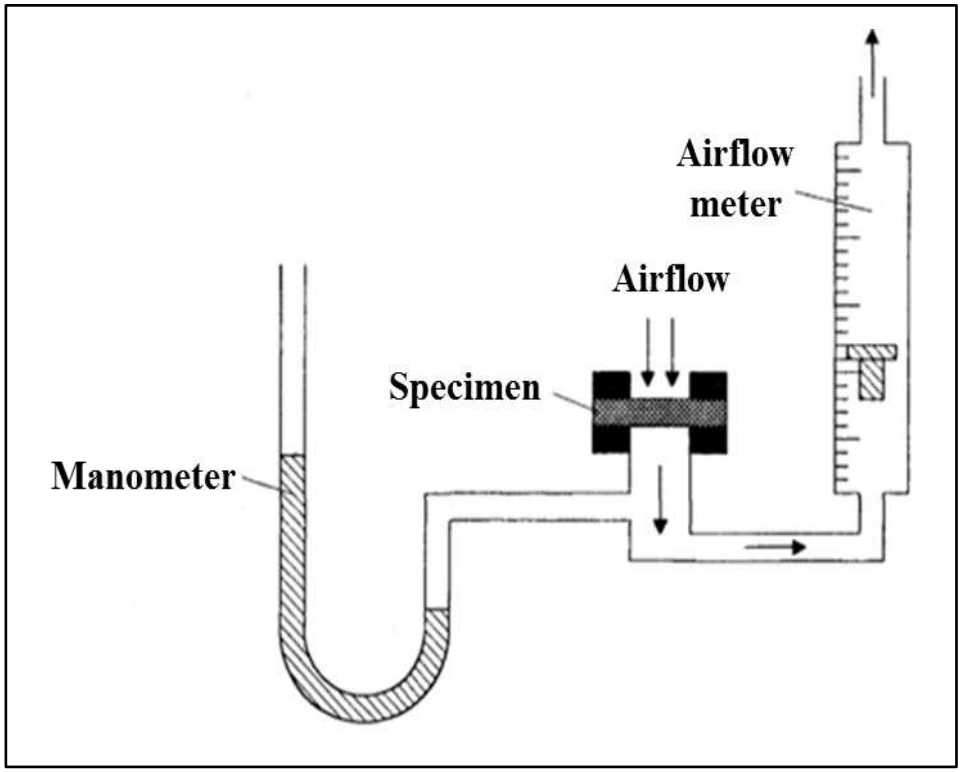


Figure 2.27: Air permeability test

I) Air permeability

It determines the fabric's ability to allow the volume of air to pass through a unit area under 10mm water level pressure. Most fabrics, including woven, nonwovens, airbag fabrics, blankets, napped fabrics, knitted fabrics, layered fabrics, and pile fabrics, can be tested using this method. Untreated, extensively sized, coated, resin-treated, etc. fabrics can also be checked. The pore properties of fabrics have the greatest impact on air movement through textiles. If there is no way or less porous fabric for air to pass through or if the airflow is problematic, it will invariably cause discomfort to the wearer. Thereby, air permeability is usually regarded as a major fabric quality parameter that contributes significantly to total clothing comfort [254].

The air permeability or breathability of nano-composite fabric used as medical textiles is regarded as a prime comfort feature and can be determined by air permeability testing. It facilitates in diagnoses any changes that occur on loading nanoparticles; change in the length of airflow pathways through fabric/composites, construction elements, etc. which can have a significant impact on air permeability [255].

Basic principle:

The fabric test area, inside the pressure difference of the fabric, measures air permeability in a specified area, in the vertical direction of the airflow rate over a specific time period. It is mostly differed as per the subjected fabric's weight, thickness, and porosity. Fabric porosity is a measurement of the air gap area as a percentage of the total fabric area. The definition is also given as 'the amount of air in cubic centimetres (cm^3) passing through cm^2 of fabric in one second with a pressure difference of 10mm head of water', also known as the Gurley unit. It is standardized by, among others, norm ASTM D737-18 and norm ISO 9237-1995.

II) Moisture Management Properties

Moisture management is a crucial feature that determines comfort apart from air permeability of medical wears e.g.; fabric, composite. Moisture management is a complex process influenced by a variety of fabric/composite characteristics, including the type of fibre (hydrophilic and hydrophobic), porosity, thickness, absorption capacity, and evaporation [256].

Table 2.6: Different types of fabric and their Properties [258]

Sr. No.	Type of Fabric	Properties
1.	Water Proof Fabric	Very slow absorption, Slow spreading, No one-way transport, No penetration
2.	Water Repellent Fabric	No wetting, No absorption, No spreading, Poor one-way transport without external forces
3.	Slow Absorbing and Slow Drying Fabric	Slow absorption, Slow spreading, Poor one-way transport
4.	Fast Absorbing and Slow Drying Fabric	Medium to fast wetting, Medium to fast absorption, Small spreading area, Slow spreading, Poor one-way transport
5.	Fast Absorbing and Quick Drying Fabric	Medium to fast wetting, Medium to fast absorption, Large spreading area, Fast spreading, Poor one-way transport
6.	Water Penetration Fabric	Small spreading area, Excellent one-way transport
7.	Moisture Management Fabric	Medium to fast wetting, Medium to fast absorption, Large spread area at the bottom surface, Fast spreading at the bottom surface, Good to Excellent one-way transport

'The controlled passage of water vapour and liquid water (perspiration) from the surface of the skin to the atmosphere through a fabric' is one of the definitions given for moisture management. One must understand both the human body's basic temperature regulation and the qualities of the textile required for this regulation. Transfer of perspiration from the skin to the atmosphere regulates body temperature and maintains thermal balance, allowing a human being to adjust the level of comfort obtained from the surroundings and activities undergone [257].

Moisture transmission through textiles has a significant impact on the human body's thermo-physiological comfort, which is maintained by perspiration in both vapour and liquid form. Perspiration must be transferred from the body, followed by quick evaporation of its fluids for keeping the body temperature in control. This requires maximising the surface area accessible for evaporation and thereby, the most effective fabrics/composites should spread moisture over a large region.

To evaluate the fabric's simple absorbency and wicking capabilities (associated with moisture management), standards and test procedures are well defined. Nonwovens can be evaluated for liquid moisture management capability or liquid strike-through time according to AATCC-195, ISO 9073-8. The Moisture Management Tester (MMT) is a device that measures the dynamic liquid transport qualities of textiles like woven, non-woven, and knitted fabrics in three dimensions. Some important definitions related to this test are given below;

- ***Absorption Rate*** – Time taken for the fabric's inner and outer surfaces to absorb moisture.
- ***One-way Transport Capability*** – Transportation of liquid moisture in one direction from the fabric's inner surface to the outer surface
- ***Spreading/Drying Rate*** - The rate at which liquid moisture spreads throughout the interior and exterior surfaces of the fabric [256].

Test Procedure:

- A fabric or test specimen is placed between two horizontal (upper and lower) electrical sensors, each with seven concentric pins, to assess the liquid moisture management properties of textiles.

- A predetermined amount of test solution is dropped onto the middle of the upward-facing test specimen surface to aid in the detection of electrical conductivity variations. The test solution can move in three different directions
- Changes in the electrical resistance of the specimen are monitored and recorded throughout this test. The electrical resistance is utilised to determine changes in fabric liquid moisture content, which is used to characterise the specimen's dynamic liquid moisture transport behaviour.
- Using interfaced computer software, a summary of the measured findings is utilised to assess the liquid moisture management qualities of any textile material.

MMT is designed to detect, measure, and record liquid moisture transport behaviour in numerous directions and it can distinguish between seven different types of fabrics (Table 2.6). To characterise the test specimen's liquid moisture management performance, a set of indexes is defined and calculated. The measurement is based on the fabric structure's water resistance, water repellency, and water absorption qualities, as well as the fabric's geometric and internal structure and wicking capabilities [258].

2.5.3.4 UV Transmission properties

UV radiation is a well-known cause for skin cancer, skin ageing, eye damage, and also susceptible to an adverse effect on the immune system. Disinfection of equipment in hospitals and labs (non-solar sources of UV radiation) are the most likely incur health consequences of medical personnel from UV radiation exposure. Properly-designed technical and administrative controls, as well as personal protective equipment in the case of hospitals and labs, can minimise these dangers to a minimum.

To determine the amount/degree of UV protection (ultraviolet protective factor) supplied by various textiles, two fundamental approaches, Vivo and Vitro are used [256]:

In-Vivo Method: This approach closely resembles the method used in evaluating the performance of sunscreen creams, i.e; determining the sun protection factor (SPF).

- In this procedure, test subjects are exposed to a standardised lamp with a spectrum of light that is as near as feasible to that of sunshine while wearing textile apparels and having an adjacent uncovered skin.

- The quotient of the time it takes for skin reddening to occur with and without textile material is then used to calculate the UPF.
- This approach requires time-consuming measurements, and the spectrum of light used for measurement is not identical to that of sunshine, making it unsuitable for designing sun-protective textile apparels.
- Filters to absorb wavelengths below 290 nm and attenuate visible and infrared light are typically employed with xenon arc solar simulators.

In-Vitro Method: This test technique requires a UV source that closely matches the sun spectrum, as well as detectors that respond similar-way to human skin, for accurate measurement. Nonetheless, for measuring relative variance in UPF, this approach is easy and effective. UV transmittance through a fabric/composite, both direct and diffuse, is a critical component in determining the UV protection of textiles material. To illuminate a fabric/composite sample, radiometric UV transmission tests employ a broadband UV light source filtered for UV-B or mixed UV-A and UV-B spectral regions. A radiometer is used to measure the total UV transmission through the material.

Because a spectrophotometer is employed, the In-Vitro method is also known as the instrumental method.

- This approach includes an in vivo component. With two primary steps: transmittance testing and calculations based on the transmittance data acquired.
- A fabric/composite swatch is placed in a spectrophotometer fitted with an integrating sphere to get transmittance data. The process is directing a beam of radiation with a specified wavelength in UV light, perpendicular to the surface of the specimen swatch and measuring the amount of radiation transmitted through the specimen.
- The transmission of radiation beams continues until all UV wavelengths (or, in some tests, wavelengths at 2 or 5 nm intervals) has been delivered to the specimen face and transmittance data have been gathered.
- Once the transmittance data is gathered (usually by measuring the UV transmittance of several swatches of the same specimen to account for variation in specimen uniformity), it is used to calculate percent transmittance values (percent UVA, percent UVB, or a total percent transmittance value, or a percent penetration value (1/UPF).

- The AS/NZS and European standards recommend that the spectrophotometer be equipped with a UVR transmission filter for wavelengths less-than 400 nm to reduce mistakes caused by fluorescence from whitening chemicals.
- At least four textile samples from a test specimen must be obtained for UPF assessment, two in the machine-direction and two in the cross-machine direction.

Table 2.7 shown the UPF ratings as per the ASTM Standards:

Table 2.7: UPF ratings [256]

UPF	Protection category	% UV radiation blocked
<15	Poor protection	<93.3
15 or 20	Good protection	93.3 – 95.9
25, 30 or 35	Very good protection	96.0 – 97.4
40, 50 or 50+	Excellent protection	97.5 – 99+

2.5.3.5 Antibacterial assessments

Medical textile performance right from the production, distribution, consumption, and disposal at all points of its serviceability heavily relies on quality. Thereby testing is obligatory for internal products and equally true for external products making direct contact with the human body. This is required to identify the chemicals or natural compounds used to add or enhance the functionality of the textile products that can adversely affect an individual's health (specifically skin) as well as the extent of harm caused. All together making testing is necessary in the case of medical textiles designed for infants and children, who are more susceptible to being affected than adults.

Plant-based antimicrobials enjoy huge therapeutic potential and have been employed since the dawn of time [259]. Practices include the use of natural products and the hunt for pharmaceuticals with a good therapeutic characteristic from plant phytochemicals, which are as old as human civilization. This is a cost-effective but safe way to treat infectious diseases [260]. That's why, from ancient time to till date mineral, plant, and animal products' importance as the primary sources of medicines remained unaltered [261].

Antibacterial-treated textiles are verified for their duration and degree of effectiveness in resisting bacteria by utilising controlled test methods capable of producing repeatable results [262]. Herbal extracts' antibacterial activity has also been evaluated in the same

pathway during the number of studies [263]. A variety of test techniques and standards are laid down for checking both qualitative and quantitative way antibacterial efficacy as desired on the practical ground [264].

I) Parallel Streak Method – AATTC 147-2004 Standard

The Parallel Streak Approach (AATCC 147-2004) is a qualitative method designed to test the antibacterial activity of diffusible and non-diffusible antibacterial agents on treated textile materials/composites that is relatively quick and easy to implement. The agar surface is infected in the Parallel Streak Method (used for diffusible agents), making it easier to distinguish between the test organism and other contaminant organisms that may be present on the unsterile specimen. The parallel streak method has been found effective in demonstrating antibacterial activity against both Gram-positive and Gram-negative bacteria over a long period of time. The antibacterial activity of non-diffusible agents can be assessed using a modified Parallel Streak Method [265].

Test Specimen:

Non-sterile test specimens are cut by hand or with a die. The sample size can be conveniently chosen but the suggested one is rectangular specimens with a cut size of 25 mm × 50 mm. Selection of 50 mm length, allows the specimens to lie across all parallel inoculum streaks [Figure 2.28(a)] ranging in width starting from minimum 8 to 4 mm broad.

Procedure:

- Pour 15 ± 2 mL of sterilised agar, which has been cooled to temperature 47 ± 2°C (117 ± 4°F), into each standard (15 × 100 mm) flat bottomed Petri disc. Before inoculating, the agar should turn to gel completely.
- To make inoculum, transfer 1.0 ± 0.1 mL of a 24-hour aged broth culture to 9.0 ± 0.1 mL of sterile distilled water in a test tube or small flask. Then thoroughly combine the ingredients by using proper agitation.
- Load one loopful of the diluted inoculum into a 4 mm inoculating loop and transfer it to the surface of the sterile agar plate. Five 60 mm long streaks spaced at 10 mm distance apart and covering the central area of the petri dish without refilling the loop is done. Precaution should be taken that while making the streaks, agar surface not been broken.

- The test specimen is gently pressed across the five inoculum streaks in a transverse direction to establish close contact with the agar surface. This is made easier by pressing the specimen to the agar surface using a biological section lifter or a spatula that has been flame sterilised and then air-cooled shortly before use.
- Place sterile glass slides over the ends of the specimen if it is susceptible to curls, preventing intimate contact with the infected surface.
- Incubate for 18-24 hours at 37 ± 2°C (99 ± 4°F).

Evaluation:

Examine the incubated plates for growth stoppage along with the inoculum streaks beneath the specimen and a visible zone of inhibition beyond the specimen's edge [Figure 2.28(b)]. Measure and calculate the average width of a zone of inhibition along with a streak on either side of the test specimen as follows to compute antibacterial efficacy.

The zone's size cannot be interpreted as a quantitative measure of antibacterial activity. Untreated matching materials and a material specimen with known bacteriostatic activity should be compared to treated materials. The observed zones of inhibition and growth under the specimen should be included in the results report. There must be no bacterial colonies directly under the sample in the contact area for satisfactory antibacterial activity [265,266].

II) Disc Diffusion Test (Agar Diffusion Test) – Swiss standard - SN195 920

The agar disc diffusion (also known as the agar diffusion test, disc-diffusion antibiotic susceptibility test, or disc-diffusion antibiotic sensitivity test) method is used to detect the presence of antibacterial or antifungal activity of the textile material (with antibacterial treatment) during antimicrobial susceptibility testing. A sterile swab is used to equally disperse the bacterial and fungal cultures over the prepared medium. The zone of inhibition is measured using a standard scale. The antibacterial activity is thus qualitatively assessed using the antimicrobial disc diffusion method [267].

Test Specimens:

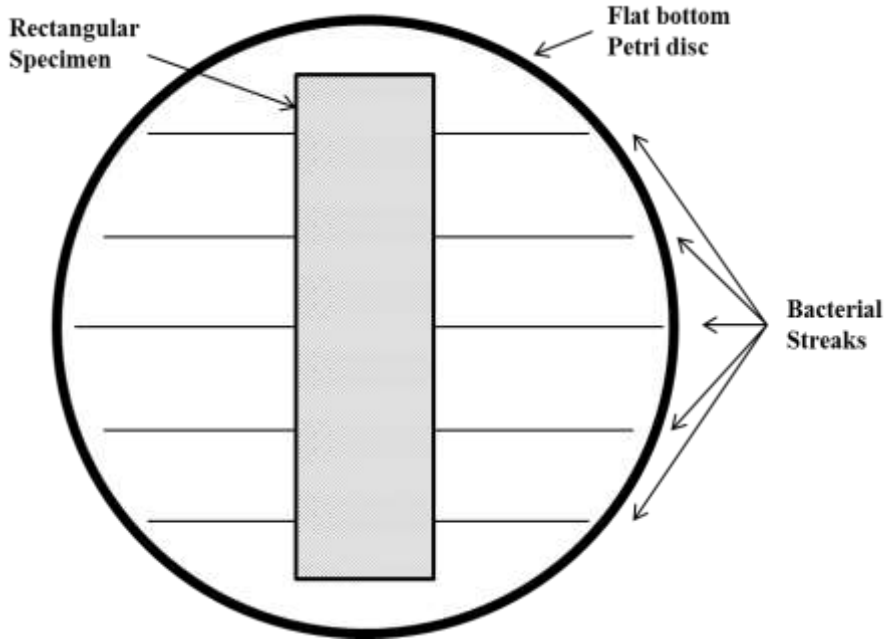
The textile material is cut into 5mm (appropriate size) diameter circular discs (antimicrobial discs) after being treated with a specific concentration of antibacterial extract.

***Procedure*:**

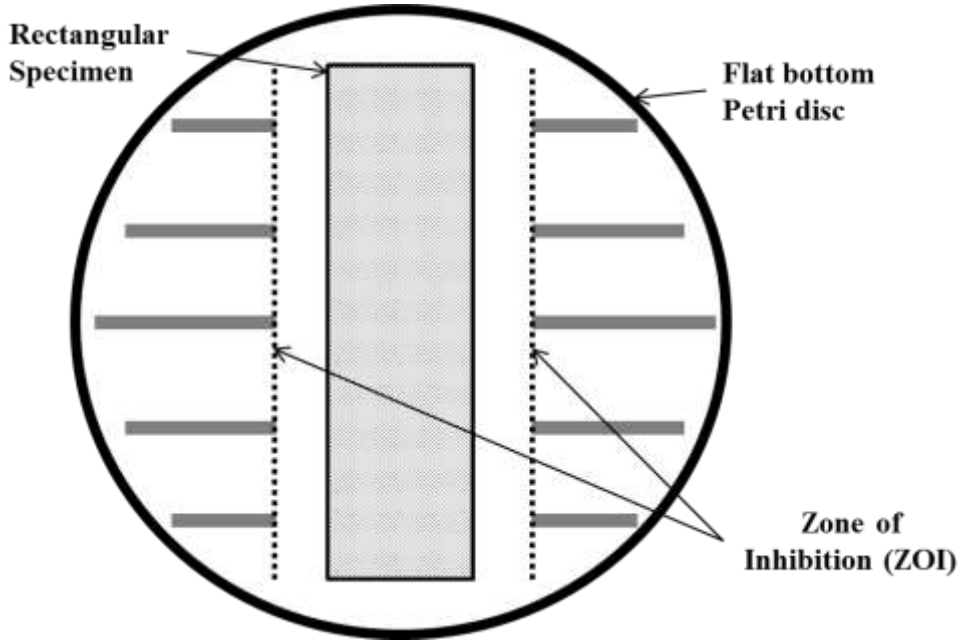
- Pour 15 ± 2 mL of sterilised agar into each standard (15 × 100 mm) flat bottomed petri dish that has been cooled to 47 ± 2°C (117 ± 4°F). Before inoculating, let the agar turn into gel completely.
- To make inoculum, transfer 1.0 ± 0.1 mL of a 24 hour aged broth culture to 9.0 ± 0.1 mL of sterile distilled water in a test tube or small flask. Using proper agitation, thoroughly combine the ingredients.
- Transfer 100 µl (0.1 ml) of this diluted bacterial inoculum and spread uniformly on the surface of UV stabilised agar Petri plates with the L-shaped glass spreader. Precaution should be taken not to break/damage the agar surface while transferring and spreading the bacterial culture.
- Label each petri dish as per the type of bacterial culture and test sample going to be loaded.
- Place sample disc (one or multiple disks) [Figure 2.29(a)] in the respective bacterial culture/s loaded agar plates with the help of sterile forceps.
    - Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location because some of the drugs begin to diffuse immediately upon contact with the agar.
    - Avoid placing disks close to the edge of the plate as the zones will not be fully round and can be difficult to measure.
- Gently press the test specimen to establish close contact with the agar surface. This is made easier by pressing the specimen to the agar surface using a biological section lifter or a spatula that has been flame sterilised and then air-cooled shortly before use.
- Incubate for 18-24 hours at 37 ± 2°C (99 ± 4°F).

Evaluation:

- After incubation, use a ruler or calliper to measure the zone sizes to the closest millimetre (mm), taking into account the diameter of the disc [Figure 2.29(b)].
- Always round up to the next millimetre when measuring zone diameters.
- If you can't read the diameter of the zone because of the disk's positioning or the zone's size, measure from the centre of the disc to a point on the zone's circumference where a definite edge is present (the radius) and multiply the measurement by 2 to get the diameter.
- A zone of 0mm can be indicated for growth up to the disk's edge [267-269].



(a)

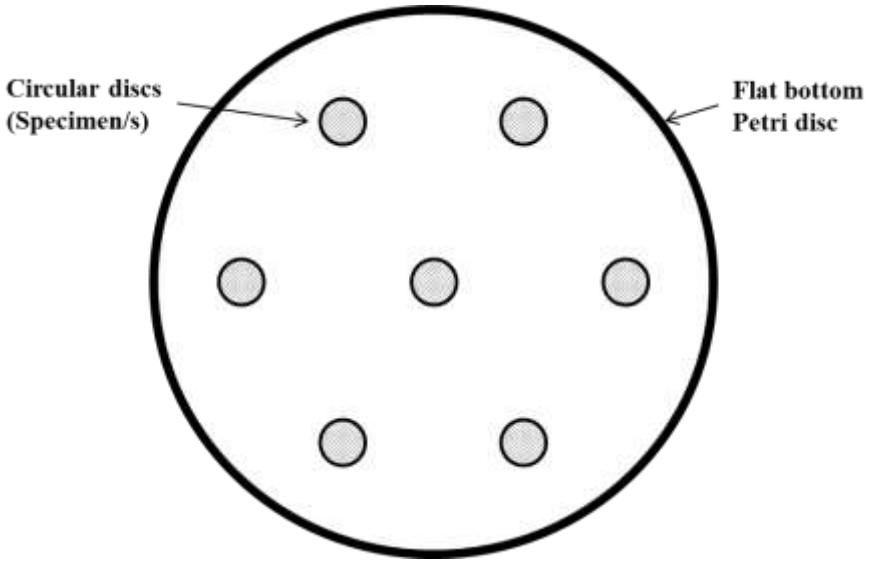


(b)

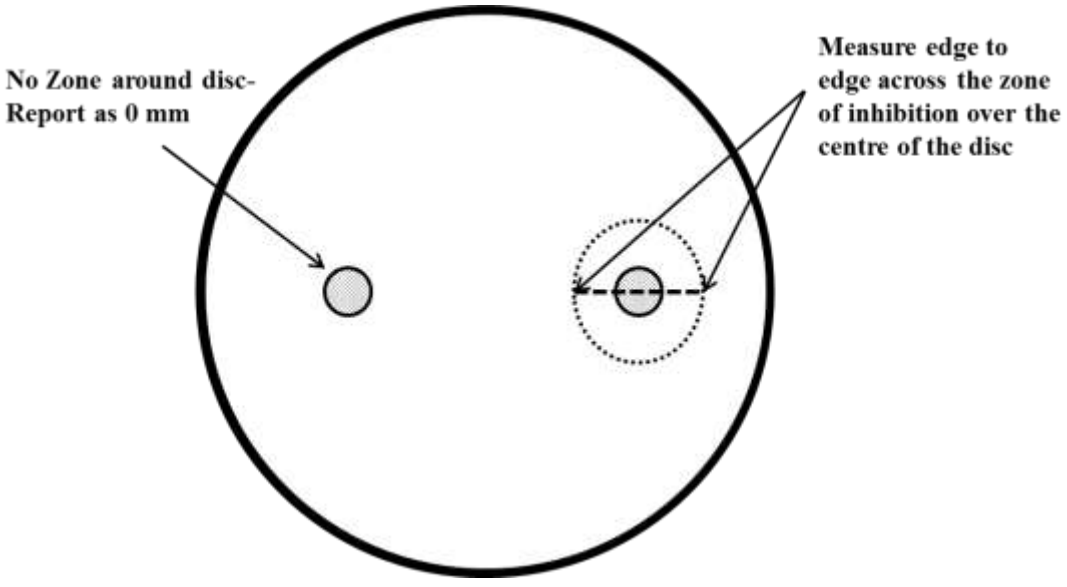
Figure 2.28:

a) Position of the test specimen in Parallel streak method, and

b) Measurement of Zone of Inhibition (ZOI) in Parallel streak method



(a)



(b)

Figure 2.29: a) Position of the test specimen in disc diffusion test, and b) Measurement of Zone of Inhibition (ZOI) in disc diffusion test

III) Various others standards for antibacterial/microbial assessments

Agar Well Diffusion Assay: To determine the presence of antibacterial or antifungal activities, antimicrobial susceptibility testing is performed using the agar well diffusion method. This approach is commonly used to assess plant or microbial extract antibacterial activity. The agar plate surface is inoculated by spreading a volume of microbial inoculum over the entire agar surface, similar to the approach employed in the disk-diffusion method. Then, using a sterile cork-borer or tip, a hole with a diameter of 6 to 8 mm is punched aseptically, and a volume (20–100 L) of the antimicrobial agent or extract solution at the necessary concentration is put into the well. The agar plates are then incubated at the appropriate conditions for the test microorganism. The antimicrobial drug diffuses across the agar medium, inhibiting the microbial strain's growth, which is subsequently assessed [270,271].

AATCC TM 100: This standard is used to evaluate textiles and fabric that have been treated with antimicrobial agents as part of the completed fabric composition, with the goal of demonstrating antimicrobial effectiveness against standard microorganisms. This test method provides a quantitative way of determining antibacterial activity levels. The degree of antibacterial activity envisaged in the use of such materials is used to assess antibacterial activity finishes on textile materials. Quantitative analysis also helps to clarify the potential applications of treated textile materials [272]. Although this test method is sufficiently sensitive, it is inconvenient and time-consuming for routine quality control and screening tests [265].

AATTC TM 30-2004*:* Antifungal Activity, Assessment on Textile Materials: Mildew and Rot Resistance of Textiles.

The following four methods are used:

- Test I Soil Burial Test – Burying cloth in fungi-infested soil is used to test fabric qualities. This process is only used for specimens that will come into direct contact with soil, such as sandbags, tarpaulins, and tents.
- Test II Agar Plate Test, Chaetomium globosum – This method is used to determine the rot resistance of cellulose-based textiles that will not come into contact with soil. It can also be used to check for fungicide treatment homogeneity.

- Test III Agar Plate Test, Aspergillus niger – Certain fungi, such as Aspergillus niger, can grow on textile goods in a laboratory experiment. Textiles are exposed to Aspergillus niger in an agar plate and fungal growth is visually evaluated when the growth of these fungi is relevant.
- Test IV Humidity Jar, Mixed Spore Suspension – Textiles are exposed to a variety of fungal spores in a humidity container. After that, any fungus growth on the textile is visually assessed [273].

ASTM D 4300: Antimicrobial testing of adhesive films to see if they can support or oppose fungi development.

ASTM E2149: Under Dynamic Contact Conditions, Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents.

ASTM E2180: Determining the antimicrobial efficacy of antimicrobial agents incorporated into polymeric or hydrophobic materials.

ASTM G21: Testing Fungi Resistance in Synthetic Polymeric Materials.

BS EN ISO 20645: Identification of antibacterial activity: Agar diffusion Plate test.

BS EN ISO 11721-1: Identification of resistance of cellulose-containing textiles to micro-organisms- Soil Burial Test – Assessment of rot-retardant finishing.

BS EN ISO 11721-2: Identification of resistance of cellulose-containing textiles to micro-organisms- Soil Burial Test– Identification of long-term resistance of a rot-retardant finish.

JIS L 1902/ISO 20743: Testing for antibacterial activity and efficacy on textile products.

JIS Z 2801: Test for Antimicrobial Activity of Plastics.

SN 195921Textile Fabrics: Identification of antimycotic activity: Agar diffusion plate test.

SN 195924 Textile Fabrics: Identification of the antibacterial activity: Germ Count Method [274].

2.5.3.6 Toxicity analysis of Plant extract, green synthesized nanoparticles, and Nano-composites

Preclinical toxicity researches done on many biological systems have been explored the investigational product's species-, organ-, and dose-specific harmful effects. Thereby, new chemical toxicity testing is said to be crucial for the innovative plant-mediated nano-composite development process. Here, toxicology denotes the performance of living tissue's reaction to non-active materials. It generally reveals the material's compatibility with the host, i.e., it defines whether the material will cause toxicity to human tissues or not while making contact with the human body. Thus, the safety of textile nano-composites, e.g. surgical non-woven materials coming in intimate contact with the human body in the forms of surgical sheets, operating clothing, and operating kits is initially manifested as toxicology or biocompatibility [275].

Histo (tissue)-compatibility and blood compatibility are the two major aspects of toxicology. The most commonly used toxicity tests are intended to investigate specific adverse events or end objectives including cancer, cardiotoxicity, and skin/eye irritation. The toxicology of compounds/composites can be determined via three routes; (a) investigating accidental exposures, (b) in vitro research utilising cells/cell lines, and (c) in vivo exposure on experimental animals [276].

According to biological evaluation of medical regarding machinery's classification standard, medical appliances, viz; surgical gown, surgical drapes, and other medical non-woven fabric/composites as a part of their surface contact, are need to be tested mainly by a toxicological test like three cell toxicity, stimulation, and sensitization.

I) Cytotoxicity test: In this method extract of the plant/substance itself and/or the material can be used, usually selected a certain extraction medium, in simulation or strict condition of clinical use of leaching. Under the condition of an experiment carried out using the selected cell lines and culture medium, the preparation test required enough new cells in order to approve at least three parallel test sample numbers and control numbers. They are representing to extract test, direct contact with the test, the indirect contact test, or membrane diffusion test. The test of cultured cells is examined with a show mirror before nearly converging and forming.

Quantitative or qualitative cytotoxicity detection methods are in use. In qualitative evaluation cells are examined under a microscope (if needed, cytochemistry staining), evaluation includes; general form, vacuolization, fall off, and the appearance of the cells. The change observed with respect to membrane integrity, generally in the form of change can be descriptive bearing in a test report or in digital records, to the meaning of test materials score Cell death, cell growth inhibition, cell reproduction, and cell clone creation all these parameters shall be determined via quantitative analysis. The number of cells, total protein, enzyme release, in vivo dye release and decrease, and all other quantitative factors shall be quantified using objective methods.

II) Skin irritation test: Animal and human skin irritation tests are covered under the skin irritation test. In most cases, an animal skin irritation test is chosen. To put it another way, appropriate animal models, with rabbits being the recommended test animals, were used to assess the material's ability to cause skin irritation under experimental settings. After removing the hair on both sides of the animal's back and spine; score and compute the accumulative stimulation index by observing the skin reaction towards the sample or its added functional extract when brought in contact with the animal's skin for a standardize test span.

III) Sensitization test: The maximal dose test and closed application test of guinea pigs are the most often utilised test procedures for delayed-type hypersensitivity response. Hence medical non-woven textiles come into direct touch with the human body, so their suitability for closed adhesive tests is defined, which assess the material's ability to cause guinea pig skin sensitization under test settings. The following criteria were used to assess the results:

1. When the level of animals in the control group is less-than one, but the level of animals in the experimental group is greater than or equal to one, sensitization is generally indicated.

2. When the level of animals in the control group is greater than or equal to 1, the reaction of animals in the test group is the most severe reaction in the control group and is considered as sensitized.