GREEN SYNTHESIS: AN ALTERNATIVE SUSTAINABLE ROUTE FOR NANOTECHOLOGY

Abstract: With the advancements and technological innovation in the past few decades, there has been an exponential increase in the nanotechnology industry. It has come to become one of the most exciting forefront fields in the 21st century. Nanotechnology is the science that deals with particulate matter at nanoscale (1-100 nm). Nanomaterials at nanoscale tend to exhibit unique properties than their bulk counterparts. The rising demand for nanoparticles and nano based products also brings in huge concerns, as the chemical and physical methods for synthesis of nanoparticles require a lot of investment and also involve toxic agents, which result it some serious after effects hazardous to both environment and the living entities. This is when green chemistry comes into the picture, green synthesis approach was found to counter the problems posed by the previous synthesis methods. The mechanism involved in green synthesis, factors affecting the synthesis and the popular techniques used in characterization of the synthesized nanoparticles are discussed in this review paper. Green synthesis has the potential to be an effective approach in minimizing the risks encountered in the traditional methods of synthesis, yet the field is new and more is to be explored and continuous research with follow up is required to adopt green synthesis at a commercial level.

Keywords: Nanoparticles, Green synthesis, Nanotechnology, Eco-friendly, Characterization

INTRODUCTION

Nanotechnology is the branch of science that deals with matter at nanoscale (1-100 nm). The nanoparticles exhibit unique properties at nanoscale that are not exhibited by their bulk counterparts. Through means of various physical and chemical processes nanoparticles with specific envisioned properties are synthesized (Chella and Thalla, 2018).The study of nanoparticles (NPs) involves a lengthy history of observation and inquiry. The evolution and growth of nanotechnology is described briefly in Table 1 (Bayda et al., 2020)

Table 1: History of nanotechnology

Year	Scientist	Contribution
1925	Richard Zsigmondy (Nobel Laureate in Chemistry)	Pioneered the theory of a nanometer Coined the term nanometer in the process of evaluating the size of gold colloids
1959	Richard Feynman (Father	At a Caltech meeting, stated “There is plenty of

	of modern nanotechnology, Nobel Laureate in Physics)	room at the bottom”, which opened up many new horizons, encouraging more research
1986	Norio Taniguchi	Coined the term “ Nanotechnology”
Early 2000’s	-	Extensive research and development in the field of nanotechnology, increased number of applications in daily life
2003	-	Concerns about nanoparticles effect on human health and environment arise
2006	-	Offshoot of green chemistry, green nanotechnology came into the picture

A study on the nanotechnology-based-products in the market by Vance *et al.*, (2015) revealed that about 1814 products containing nano-sized materials were present in the global market, introduced by approximately 622 companies. At present there exists to be a huge demand for nano-sized materials which adds to be near about 300,000 to 1.6 million tonnes worldwide. The Asia-Pacific region holds the largest market share of about 34 per cent, which is followed by North America (31%) and Europe (30%).

On the other hand, with the rise of nanotechnology huge concerns are also on the rise related to their impact on the environment and toxicity of the nanoparticles on the living entities (Buzea*et al.*, 2007). Furthermore their possible influence on the global economics, along with a hypothetical doomsday theories. Hence due the concerns on rise, it has led to the debate among advocacy groups and governments on if special regulations on nanotechnology are obligatory.

SYNTHESIS OF NANOPARTICLES

Nanoparticles are synthesized by two distinguishing approaches. One is the “top-down approach” and the other is the “bottom-up approach” (Iqbal *et al.*, 2012). In top-down approach, solid bulk matter is broken down into smaller particles and further reduced to nano-sized particles using mechanical forces such as ball milling, laser ablation, etc which help in breaking down the particles to nano-size and later the nanoparticles are stabilized to required size (Lu *et al.*, 2020; Unal *et al.*, 2020). But, it is difficult to breakdown to achieve the required nano-size using top-down approach. And the bottom-up approach starts with matter at atomic level, where they use chemical (hydrothermal method, sol-gel method, gas phase method, thermolysis, and hydrolysis) and biological routes to assemble the atoms to initiate new nuclei, thereby fabricating the particles to grow to the required nanosize at the end of the synthesis (Varadan *et al.*, 2010).

Top-down approaches demand huge capital involving high costs, high amount of energy and is time consuming, even after which there is no guarantee of achieving the desired nano-size at the end of the synthesis. And though some top-down approaches involving UV irradiation, laser ablation, aerosol technologies and photochemical reduction processes produce nanoparticles, they also release some harmful byproducts during the synthesis. And further the there is no control over the surface chemistry, size, and structure of the nanoparticles in top-down approaches. Therefore bottom-up approaches are often preferred for synthesis which begin with simple molecules that are later grown into nanoparticles and also allows a greater control upon the shape and size of the nanoparticles to be synthesized. Even though chemical synthesis has certain advantages that include less time consumption, low cost equipment, low space and temperature requirements, the chemicals used for the synthesis lead to generation of harmful and toxic by-products that affect the environment and living beings (Chella and Arun, 2018).

In order to counter these limitations, since the past decade a new era of green synthesis has gained great interest among the researchers (Santo-Orihuela*et al.*, 2022). The traditional methods (physical and chemical) of synthesis of nano-sized materials demanded sophisticated equipment under extreme harsh conditions. But it contrast, the green route of synthesis of nano-sized materials is carried out under ambient temperature and pressure, which suggests simplicity, energy and money saving. Essentially, green synthesis of nanoparticles fabricated by proper regulation, control, clean up and remediation process directs its efforts towards environmental friendliness.

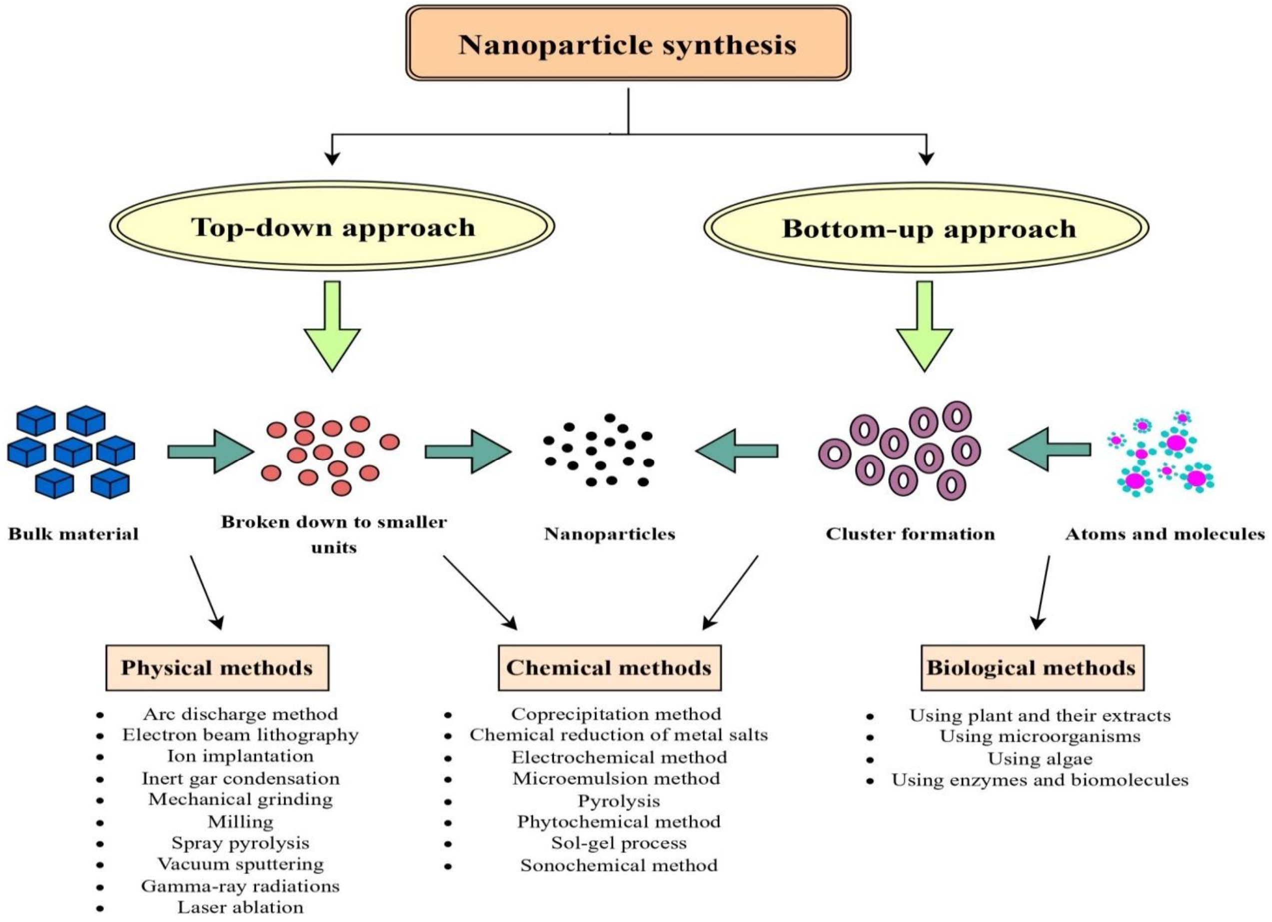

Fig 1: Top-down and Bottom-up approaches for nanoparticle synthesis

GREEN SYNTHESIS

Scientists can no longer assume green science or green route to be just an option, but the most preferred option. With the increasing synthesis of nanoparticles, so is the need for new approaches for the synthesis so as to cause less harm to the biosphere. In the early 2000s Anastas and Warner in their book “Green Chemistry” established the basis of green chemistry, which consists of 12 basic principles (Anastas and Warner, 2000). They include:

1. Prevention – Steps should be devised to prevent or minimize waste production.
2. Atom Economy - Too carry of the synthesis with as much as minimum materials.
3. Less Hazardous Chemical Synthesis - Prioritization of methods that result in minimal or no toxicity.
4. Designing Safer Chemicals - Chemicals should be designed such that they show limited or no toxicity.

5. Safer Solvents - The use of solvents and auxiliary chemicals needs to be avoided or minimized wherever feasible.
6. Design for Energy Efficiency - Efficient energy utilization and saving methods need to be adopted.
7. Use of Renewable Feedstocks - renewable feedstock and depletion should be avoided whenever possible.
8. Reduce Derivatives – Additional wastes generated through derivatives such as blocking agents and protecting/deprotecting groups need to be avoided whenever possible.
9. Catalysis - Catalysis agents are desirable than the stoichiometric agents.
10. Design for Degradation - Chemicals should be designed in such a way that at the end of synthesis, they break down into non-toxic derivatives.
11. Real-time Analysis for Pollution Prevention - Synthesis should be monitored in realtime to check for toxic chemical production.
12. Inherently Safer Chemistry for Accident Prevention - Selection of agents in synthesis should be such that the possibility of hazardous accidents is nill or reduced to the maximum extent possible.

These 12 principles are taken into consideration in green chemistry whenever feasible, in order to contain the release of hazardous chemicals into the environment and limit the human exposure to those chemicals (Anastas and Warner, 2000). Galuszka *et al.*, in 2012, expanded these 12 principles in their review paper review of green analytical chemistry and also proposed the mnemonic “SIGNIFICANCE” as an easy means to memorize the 12 Principles of Green Chemistry.

- S - Select direct analytical technique
- I - Integrate analytical processes and operations
- G - Generate as little waste as possible and treat it properly
- N - Never waste energy
- F - Implement automation and miniaturization of methods
- I - Increase safety of operator
- C - Carry out in-situ measurements
- A - Avoid derivatization
- N - Note the sample number and size should be minimal
- C - Choose multi-analyte or multi-parameter methods
- E - Eliminate or replace toxic reagents

The scientists were well aware of the fact that the biological entities possessed the ability of reducing the metal precursors in the late nineteenth century itself, yet its mechanisms were vaguely explored during that time. Eventually later with positive research progress of the green synthesis they were able to harness the power of renewable sources like plants and microbes, which could act as reducing agents thereby stabilizing and capping the nanoparticles,(Bandeira *et al.*, 2020; Galdopórpora *et al.*, 2021) rather than the use of toxic, expensive chemicals and techniques that involve high energy consumption, the researchers were more attracted towards the biological methods (Luangpipat *et al.*, 2011; Dhillon *et al.*, 2012; Arumugama *et al.*, 2015;Santo-Orihuela*et al.*, 2022). Conventional methods for the synthesis of nanoparticles are more in practice at industrial scale past many years, but the green route has been proven to be quite effective for synthesis of the nanoparticles owing to its low cost, ease of characterization and less chance of failure (Bhalerao and Borkar, 2017; Abdelghany*et al.*, 2018).

Green synthesis offer several advantages over the traditional methods (Moosa *et al*. 2015; Koul *et al.*, 2018; Singh*et al.*, 2019) :

1. It is pollution free (Alsammarraie *et al.*, 2018)
2. It is cost effective and economical (Kataria and Garg, 2018).
3. It is environment friendly.
4. Feasibility of huge amount of synthesis with advancing technologies.
5. No necessity of sophisticated equipment or need of toxic materials (Devi *et al.*, 2019).
6. More sustainable (Nasrollahzadeh and Sajadi, 2016).

Accordingly green synthesis of nanoparticle emphases on three key aspects (Mohan *et al.*, 2014):

- A green solvent
- An eco-friendly benign reducing agent
- A nontoxic material as a stabilizer

The extracts from leaves (Logeswari *et al.*, 2015; Chahardoli *et al.*, 2018; Leili *et al.*, 2018; Devi *et al.*, 2019), flowers (Thovhogi *et al.*, 2016, 2015; Sone *et al.*, 2020), roots, peelings (Ehrampoush *et al.*, 2015), fruits (Kumar *et al.*, 2017), and seeds (Dhand *et al.*, 2016; Gao *et al.*, 2016) of various plants or microorganisms (bacteria, fungi and algae) (Subramaniyam *et* *al.*,2015; Arsiya *et al.*, 2017; Saravanan *et al.*, 2018) are being used at present for the synthesis of the nanoparticles through the green route as shown in Table No.2 (Salem and Fouda, 2021).For green synthesis of nano-sized components both microbes and plant mediated approaches are in practice. In the synthesis involving microbes, the in-built sophisticated biochemical mechanismsare put into action for reducing of the ions into nano-sized particles, it often leads to well-defined nanoparticles varying in compositions, shapes and sizes accordingly (Antezana et al., 2022). But when a large scale production is in question, the synthesis involving microbial preparations is challenging. But this could easily be tackled by using plant based extracts for synthesis, where the production rate could be significantly amplified.

Table 2: Different biological agents involved for biosynthesis of nanoparticles

Biological source	**Factors that aid in synthesis**	**Advantages**	**Disadvantages**
Plant	Secondary metabolites act as capping and stabilizing agents	Minimal cost, eco-friendly, no sophisticated equipment required, low energy consumption, lack of toxic precursors, biocompatibility, no extra efforts for culture or colony maintenance	Not fully explored yet the after effects or harmful effects, the low yields of secreted proteins generated by plants decrease the rate of synthesis
Fungi	Reducing enzymes and biomimetic mineralization	Large scale NP fabrication, low cost, eco-friendly, low energy demand, increased metal accumulation, enhanced wall-binding capacity, simple biomass handling	Low reproducibility, problematic genetic manipulation, other solvents required for obtaining pure NPs, particle size distribution is broader
Algae	Polysaccharides act as capping and stabilizing agents	No toxic byproducts, biocompatible, can grow under diverse conditions, cost effective, easy handling, no cellular maintenance required	Algal culture demands more time, more work on reproducibility needed, size control is limited, Up-scaling fabrication is limited, not all species can be used in NPs synthesis
Bacteria	Nitrate-dependent reductase or NADPH-dependent reductase enzymes in bacteria	Non-toxic, cost effective, energy saving	Tedious procedures isolation, sampling, storage and culturing involved, morphology of

reduces metal ions	NPs is difficult to control

The plant extracts are rich in compounds that possess the ability to reduce complex metal ions into simple ions. In fact the idea of the plant extracts for the metal ions reduction to nano-sized materials was inspired by the accumulation of metallic ions in plant cells and tissues (Das *et al*., 2012). The production rates are proficient in case of plant extracts mediated synthesis when compared to the microbes mediated synthesis. The plant extracts have been proven to be able to reduce the metal-ions at a faster rate that the microbial entities, and are also known to produce nanoparticles that are very much stable (Ravichandran, 2010; Vance et al., 2015). Though there are relatively diverse methods followed during green synthesis, the chief function that happens is that the biological agents utilized in the synthesis reacts with different metal salts in the reaction mixture, thereby reducing them to nanoparticles, which are later used for various purposes but only after characterization (Catalano *et al*., 2021; Kagdi *et al*., 2022).The compounds like alkaloids, falvonoids, phenols, tannins etc., present in the plant extracts have shown the ability to reduce the metal ions to nanoparticles with good stability (Makarov et al., 2014; Krestinin et al., 2015). While in case of microbes mediated synthesis the important enzymes present in them serve as a reducing as well as stabilizing agents during the nanoparticle synthesis (Ovais et al., 2018).

The green raw materials containing different enzymes/proteins,polysaccharides, vitamins amino acids,poly- phenols, etc., (Can, 2020), have the potential to act as both reducing agents where they reduce the metal ions to a stable state from the excited state and capping agents thereby replacing the chemical reagents in nanoparticles synthesis(Collera-Zúñiga *et al*., 2005; Afreen *et al*., 2020). With proper favorable conditions (temperature, concentration, ambient air and others) green synthesis successfully yields nanoparticles and also surpasses the nanoparticles synthesized through chemical methods.

MECHANISM OF SYNTHESIS OF NANOPARTICLES

Even though the key aspects that draw our attention towards green synthesis are its cost effectiveness and environment friendly nature, it is the stability of the green synthesized nanoparticles that has grabbed the interest of the researchers (Trickler*et al*., 2010). It was reported that at high extract concentrations, the biomolecules that act as reducing agents, also cover the nanoparticle surfaces, thereby preventing them from aggregation and increasing their stability (Khalil *et al.*, 2014). Though the synthesis of nanoparticles through the green route is now practiced widely, yet the mechanism of their synthesis poses as a challenge for the scientists (Velusamy *et al.*, 2016).

The formation of nanoparticles is completed in primarily three phases:

- Ion reduction
- Cluster formation
- Growth of nanoparticles

In technical terms the synthesis of nanoparticles from plant extracts happens in three phases:

- Activation phase
- Growth phase
- Process termination phase

The activation phase of the synthesis is the phase where the reduction of the metal ions takes place, which further leads to formation of new structures through nucleation (self-organization) of the reduced metal atoms. Further in the growth phase (second phase) the newly formed structures grow, with additional metal ions reduction along with increased thermodynamic stability of the formed nanoparticles. In the final stage, which is the process termination phase, the final shape of the nanoparticles is attained and the shape of the nanoparticles formed affects their stability (Chella and Thalla, 2018).

For instance when we talk about synthesis of nanoparticles from plant extracts, a standardized method involving systemic approach is followed. Careful selection of the specific plant along with its taxonomical identification is done, after which plant extract from the desired plant part is extracted using appropriate solvent/s, which is followed by some purification techniques like filtration/chromatography/centrifugation to get rid of any impurities. At the same time the required metal salt solution is prepared which acts as the nanoparticle precursor and the earlier plant extract prepared is added to this metal salt solution, during which the optimum temperature and pH for the reaction to happen are maintained accordingly, that would help in initiation of the reaction leading to the formation of the nanoparticles (Safat*et al.*, 2021). Among the several methods studied, better results were obtained when there was continuous stirring of the reaction mixture, which resulted in formation of uniform-sized nanoparticles that is indicated visually by a noticeable colour change. Additionally ultrasonic treatment application helps in ensuring uniform dispersion of the synthesized nanoparticles in the solution. After which the synthesized nanoparticles are separated from the solution through centrifugation, where subsequently they are washed to get rid of any remaining impurities. As per preference the nanoparticle precipitate obtained can be further dried using hot air oven or with 70 % ethanol at room temperature to eliminate any additional impurities (Alhujaily*et al.*, 2022).

FACTORS AFFECTINGSYNTHESIS OF THE NANOPARTICLES

The features like shape, size and quality of the biosynthesized nanoparticles depend on wide range of aspects such as the plant extract concentration used, metal ion solution concentration, composition and pH, also the temperature at which the reaction takes place (Mittal *et al.*, 2013; Shah *et al.*, 2015; Rautela *et al.*, 2019). The factors affecting the synthesis of nanoparticles are described below in Table3.

The prime factors responsible for size variations among the synthesized nanoparticles could usually be:

- Polyphenols concentration (Nadagouda *et* al., 2010; Huang *et al.*, 2014), as they play a crucial role as reducing and capping agents in synthesis.
- pH of the reaction mixture, it is because acidic and alkaline pH can cause agglomeration among the nanoparticles due to over nucleation (at low pH) and instability of nanoparticles (at high pH).

The size and shape of the nanoparticles during synthesis can be easily regulated by monitoring the parameters like pH and temperature of the initial reaction mixture.The pH variation strongly influences the nucleation process. Across various studies it has been reported that when synthesis of nanoparticle was carried under acidic pH conditions, it usually resulted in formation of nanoparticles with poor stability. But in case of alkaline pH conditions the synthesis process happened at a faster rate along with production of nanoparticles with good stability. In acidic (low pH) conditions, the growth of the nanoparticles is known to be in the form of clusters which would eventually agglomerate, while in case of higher pH (alkaline) conditions, many pearl-like nanoparticles formation was reported along with some large diameter nanoparticles during the synthesis (Shou *et al.*, 2011). And in case of temperature, faster growth dynamics was observed at higher temperatures (more than normal room temperature) but at the same time, when the reaction rate is faster defects were observed thereby affecting the crystalline structure and quality of the synthesized nanoparticles. The nucleation time is also critical in controlling the size and size distribution ofthe nanoparticles, smaller nucleation time usually provides a better control over the size of the nanoparticles synthesized.

Table 3: Factors affecting the size and shape of the nanoparticles during synthesis

Sr. No.	Factor (s)	Effect on NPs synthesis
1	**Technique or Method**	The nanoparticles can be synthesized in numerous ways using physical, chemical and biological means. The green route of synthesis being economical, non-toxic and eco-friendly is more advantageous over the traditional methods (Otsuka*et al*., 2003; Prasad*et al*., 2011).
2	**pH**	Nanoparticles tend to show aggregation and are less stable in pH (acidic) conditions ranging from 3 to 6, while in case of pH lower than 3,the nanoparticles are more stable as the protonated forces of all the molecules work against electrostatic interactions. Likewise, in conditions with pH above 7 due to deprotonation, the repulsion of aggregated molecules is seen.
3	**Temperature**	The physical approaches demand high range of temperature more than 350^{o}C, and it is not that higher in case of chemical approaches. But in contrast to these methods, slightly higher temperatures (but below 100^{o}C) than the normal room temperature is sufficient to carry out the synthesis by green route. The nature and size of the synthesized nanoparticles can be regulated by the reaction temperature (Chauhan*et al*., 2012).
4	**Pressure**	Pressure is one of the important factors during the synthesis. At ambient pressure, in green synthesis the metal ions are reduced at a faster rate (Pal*et al*., 2011).
5	**Time**	Time is another significant factor that affects the rate of synthesis of nanoparticles through green synthesis (Brice-Profeta*et al*., 2004; Kowalczyk*et al*., 2011). Shrinking and enlargement of the nanoparticles could happen within the synthesized nanoparticles when stored for a prolonged period of time, ultimately affecting their functional properties. The aggregation among the nanoparticles is also influenced by the synthesis process and the storage conditions (Brice-Profeta *et al*., 2004; Faraji*et al*., 2010; Priya*et al*., 2011;Rajeshkumar*et al*., 2016).
6	**Particle shape and size**	The size and shape of the nanoparticles greatly influence the synthesis and the functionality of the nanoparticles. Usually the size reduction of the nanoparticles also brings down the melting point of the synthesized nanoparticles (Priya *et al*., 2011).
7	**Pore Size**	Nanoparticles quality and its applications are both affected by the porosity of the formed nanoparticles. Generally this factor is exploited for drug delivery, where the desired molecules of biological origin are bound to the surface of the nanoparticles for specified objective (Molpeceres*et al*., 2000).

Optimization of the solvent ratio, reaction time, temperature, pH and the ratio of the plant extract to the metal solution (reaction mixture) are all the crucial aspects that needs to addressed during green synthesis of the nanoparticles. Yet, still because of the involvement of various biomolecules that act as reducing, stabilizing and capping agents the interaction between the polyphenols and nanoparticles is not very clear. The surface charge of the nanoparticles is one another important parameter for its characterization. The nature and intensity of the surface charge influence the interaction between the nanoparticles and the biological environment (the bioactive compounds from plants, algae, fungi, and bacteria).

The stability of the nanoparticles is one of the vital requirements as the synthesized nanoparticles later have a vast range of applications.

CHARACTERIZATION OF THE NANOPARTICLES

In the end using diverse analytical techniques the synthesized nanoparticles are characterized in order to estimate their composition and physiochemical properties. They provide essential insights with respect to the properties and behavior of the synthesized nanoparticles. For instance to configuration of shape, size and structure of the synthesized nanoparticles Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) are employed, while the optical properties are assessed using UV-Vis spectrophotometry and to identify the functional groups present on the surface of the nanoparticles FTIR spectroscopy is used. Furthermore Dynamic Light Scattering (DLS) and Zeta Potential measurements are employed to estimate the size and surface charge of the nanoparticles (Khane*et al.*, 2022; Mahmood*et al.*, 2022; Alzubaidi *et al.*, 2023). Many other techniques and instruments used for characterization of the nanoparticles are described in Table 4 below.

Table 4: Techniques and instruments used for characterization of nanoparticles

Objective	Instrument/ Technique	Purpose	References
Formation of Nanoparticles	UV (Ultraviolet-visible) spectrophotometry	To estimate size, structure, stability of nanoparticles including their aggregation	Otsuka *et al.*, 2003; Tiwari *et al.*, 2008; Parida*et al.*, 2011; Talam*et al.*, 2012; Chandra*et al.*, 2014
Size and morphology of NPs	TEM (Transmission electron microscopy)	To evaluate the morphology (size and shape) along with the structural allography of NPs	Chauhan *et al.*, 2012; Gupta *et al.*, 2013; Arakha*et al.*, 2015; Poguberovi*et al.*, 2016
	High TEM (Transmission electron Microscopy)	For determining atom's arrangement and local microstructures	Brice-Profeta *et al.*, 2005; Kowalczyk *et al.*, 2011; Thomas*et al.*, 2013;Choi*et al.*, 2014
	SEM (Scanning electron microscopy)	Morphology examination of the nanoparticles	Faraji *et al.*, 2010; Das *et* al., 2010; Priya*et al.*, 2011; Luo *et* al., 2016; Rajeshkumar*et al.*, 2017
	AFM (Atomic force microscopy)	To estimate the size, morphology or surface texture	Molpeceres *et al.*, 2000; Gupta *et al.*, 2013; Nithya *et al.*, 2014; . Logeswari*et al.*, 2015
	DLS (Dynamic Light Scattering)	Evaluation of the particle size distribution	Molpeceres *et al.*, 2000; Chauhan *et al.*, 2012;Salunke*et al.*, 2014 Rajeshkumar *et al.*, 2017

Surface study or Charge on the surface of NPs	Zeta potential	Determination of the charge on the surface and stability of the NPs	De Jaeger *et al*., 1991; Otsuka*et al*., 2003
	FT-IR (Fourier-transform infrared-spectroscopy)	Characterization of the functional groups present on the surface of the nanoparticles	Otsuka *et al*., 2003; Brice-Profeta *et al*., 2005; Janaki*et al*., 2015; Dobrucka *et* al., 2016; Rajeshkumar*et al*., 2017
	XPS (X-ray photoelectron spectroscopy)	Characterization of the bonds involved and determination of the mechanism of the reactions occurring in the nanoparticles surface	Brice-Profeta *et al*., 2005; Faraji *et al*., 2010
	Thermal gravimetric analysis	To estimate the binding efficiency of the coating on the nanoparticles surface	Brice-Profeta *et al*., 2005; Faraji *et al*., 2010
Crystallinity	XRD (X-ray diffraction)	To determine the crystalline structure of the nanoparticles	Molpeceres *et al*., 2000; Tiwari *et al*., 2008; Chauhan *et al*., 2012; Umer*et al*., 2014; Wang*et al*., 2014
Magnetic properties	VSM (Vibrating sample magnetometry)	To determine the magnetization of the magnetic nanoparticles	Brice-Profeta *et al*., 2005; Faraji*et al*., 2010
	Superconducting-quantum-interface device magnetometry	Confirmation of the magnetic properties of nanoparticles	Brice-Profeta *et al*., 2005; Faraji *et al*., 2010
	Field flow flotation	Separation of nanoparticles based on their magnetic susceptibility	Vickrey*et al*., 1980, Kowalczyk *et al*., 2011

Others	Chromatography	Separation based on mobility/ affinity of nanoparticles	Alves *et* al., 2009; Chauhan *et al*., 2012; Hossain *et al*., 2013; López-Serrano *et al*., 2013
	X-ray spectra (Energy dispersive)	To evaluate the elemental composition of the nanoparticles	Otsuka *et al*., 2003; Lal *et al*., 2011
	Centrifugation	Separation of nanoparticles based on their density	Bootz *et al*., 2004; Balnois *et al*., 2007; Mavrocordatos *et al*., 2007
	Laser-induced breakdown detection	To estimate the size and colloidal conentration	Bundschuh *et al*., 2001 (a), 2001(b)
	Mass Spectroscopy	To determine size and charge state, depth profiling in fluorescent labelled nanoparticles	Cai *et al*., 2003; Salunke*et al*., 2014
	X-ray fluorescence spectroscopy	Quantification of elemental concentrations in powdered/liquid nanoscale samples	Tiede *et* al., 2008; Lo´pez-Serrano*et al*., 2014
	Small angle X-ray scattering	Determine the structural characteristics of nanoparticles	Tiede *et* al., 2008; Lo´pez-Serrano *et al*., 2014
	Energy dispersive X-ray spectra	Evaluation of the elemental composition of the nanoparticles	Herguth*et al*., 2004; Prasad*et al*., 2011
	Hyperspectral imaging	Assessing nanoparticles type, estimation of the fate and transformation of the nanoparticles in the water samples and also determining the surface chemistry along with functional groups added to the nanoparticles	Badireddy*et al*., 2012

CONCLUSION

Possessing the unique qualities that the nanoparticles possess, they have a varied range of applications. And so is the field and application of nanomaterials is escalating, therefore extensive research is now thus focused towards studying about the synthesis, their characteristics, behavior and the wide range of applications and ultimately their effect in the environment.

The traditional methods of synthesis pose a huge threat to the environment because their high energy requirements and the toxic reagents involved. Carrying on with the traditional methods of synthesis even further in the future indicates both our negligence and ignorance of the distressing after effects towards the environment. And hence demands for a more conscious approach.

The green route for the synthesis of nanoparticles is a simple process which is cost effective and eco-friendly too, that requires not much effort or time. And most importantly it causes minimal or no harm to the environment or the living entities. At the same time, green synthesis has its own set of drawbacks, while nanotechnology has already raised the stakes for human health, the limited studies on the bioaccumulation and toxicity of the nanoparticles in the environment is a huge concern that is mounting upon. Due to their small size, nanoparticles pose a constant threat, as they are prone to cause inhalation problems and many other fatal diseases. And at commercial level green synthesis of the nanoparticles has yet not been explored and is long due.

So addressing the huge potential applications of the nanoparticles in various fields, according to feasibility of cost and techniques green mode of synthesis can be adopted. Furthermore, a clear understanding of the different biochemical pathways that take place during the synthesis would allow for the adaptation and evolution of the sustainable green routes for the synthesis of nanoparticles at a larger scale. And it is also important to note that the pros and cons of green synthesis can vary depending on the specific method, reaction and desired outcome. Therefore continuous research and disclosure of the developments and findings in this field is a must to augment the applicability and effectiveness of green route for synthesis of nanomaterials.

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