CHAPTER 1

1.1 Introduction

Nanomaterials have gained tremendous scientific attention due to their versatility and unique properties in numerous fields; especially, they become powerful tools in biomedical research and applications [1]. Their multimodal properties and versatile applicability make them indispensable tools in several technologies, such as therapeutics, bioimaging, nanomedicine, and mechanistic understanding of biological systems [2]. Despite having great potential in the biomedical area, the real-time application of nanomaterials is still limited due to biocompatibility issues, low scalability, poor reproducibility, regulatory consideration, non-degradable by-products, small systemic clearance, and short-term stability [3,4] Therefore to address above challenges, there is an urgent need to design novel approaches to overcome the limitations and harness its full potential in the area of bioimaging, biosensing, antimicrobial activity, drug delivery and theragnostic [4]. However, for real-time application of nanomaterials, their small size and thermal stability are of great importance [5]. Despite great advancement in the area, there is scanty work related to the thermodynamic and kinetic stability of nanomaterials [6]. Therefore, there is an urgent need to develop deep insight into the fundamental scientific understanding pertaining to the computation of thermodynamic parameters, nucleation rate, and interfacial energy [7,8]. A forecast of these parameters using computational methods will significantly reduce the number of experimental steps and provide significant advantages in various applications such as catalysis, nanomedicine, polymer design, and optoelectronics [9]. The current thesis, therefore, plans to synthesize Alkali earth metal-based nanomaterials using the one-pot facile reduction route and investigate them for

obtaining properties such as biocompatibility, fluorescence imaging, angiogenesis, and antimicrobial activity. Furthermore, current thesis undertakes to study thermodynamic parameters such as free energy, enthalpy, interfacial energy and nucleation rate to estimate the thermodynamic stability of the developed nanomaterial.

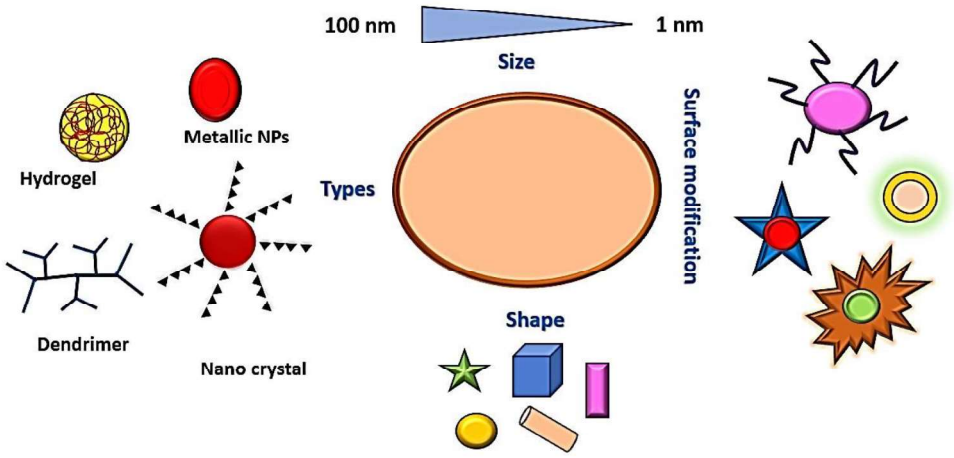


Figure 1.1: Schematic representation of nanomaterial-based on size, shapes, types, and surface modification.

1.2 Synthesis strategy of nanomaterials

Numerous physical and chemical methods exist for the production of nanoparticles [4] .For nanoparticles (NPs), physical processes frequently employ a "top–down" strategy involving the subdivision of a precursor material into smaller units [1]. In contrast, biogenic strategies are referred to as 'greener synthesis' because they are typically more cost-effective, environmentally benign, less toxic, having high bio reducing efficacy ,biologically safe ,manageable and easy available [10,11].

These routes involve the extraction of bio-reductant from plants, bacteria, fungi, and other natural resources. Whereas, chemical procedures are predominantly "bottom–up" approaches that begin with a chemical reaction delivering metal atoms, accompanied by the controlled aggregation of atoms into particles. Predominately, chemical processes (e.g., reduction, thermal decomposition, or sol–gel synthesis) are more suitable for obtaining small and uniform particles [12,13]. For these syntheses, metal nanoparticles (Au, Ag, Pd, Pt, Cu.) are typically prepared in aqueous or non-aqueous solutions by reducing a dissolved metal precursor (typically a metal salt) with a reducing agent such as sodium borohydride, ascorbic acid, trisodium citrate, or phenols [14,15]. This process is frequently conducted in the presence of a stabilizing agent, which affects the aggregation behavior of clusters and NPs. Stabilizing agents adsorb on the surface of NPs and provide, via repulsive forces, electrostatic stabilization (such as citrate ions) or steric stabilization [16] (as with polymers such as PVA (poly (vinyl alcohol)) and PVP (poly(vinylpyrrolidone)). In addition, organic ligands with a significant affinity for metal surfaces through phosphine or thiol functionality can inhibit NPs aggregation [17]. Thiols such as disulfides, polymers with mercapto groups, and dendrimers are used as capping agents. Micelles or emulsion particles can be utilized as nanoreactors that confine the precursor solution locally; consequently, spatial separation precedes NPs formation [18].

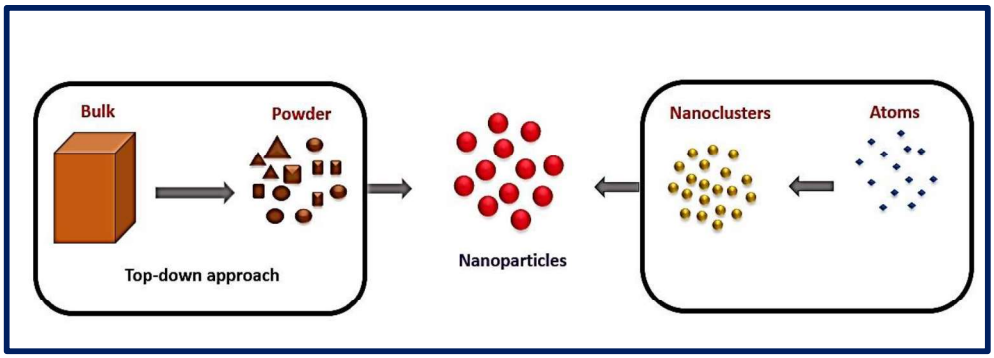


Figure 1.2: Schematic representation of top-down and bottom approach for the synthesis of nanomaterial.

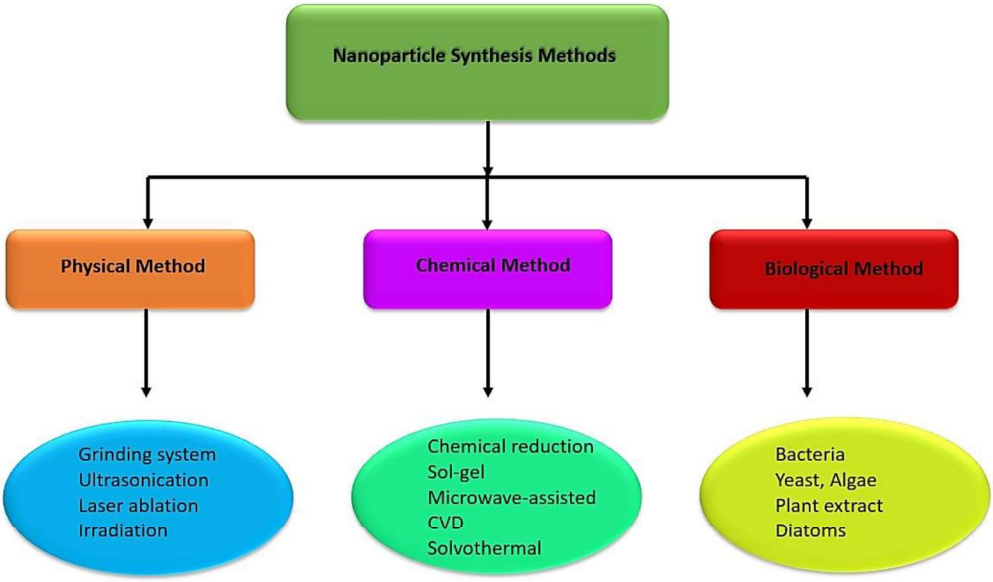


Figure 1.3: Flow diagram of different methods applied for the synthesis of nanomaterial.

1.3 Size control of nanomaterials

The size of NPs can be controlled by thermodynamic, kinetic, and stoichiometric methods [19]. Strategies can rely on a firmly adsorbed stabilizer on the NPs surface, arresting a

limited number of reactants in micelles (i.e., stoichiometric control), restricting additional "nucleation events" (e.g., seeded-growth), or subsequent treatments that increase NPs size homogeneity Oswald ripening. Utilizing capping agents that typically adsorb on the nanocrystal surface to form a nanoparticle–stabilizer entity, which then represents a thermodynamically stable NPs configuration, is a common method for controlling particle size. As reported for numerous metal nanoparticle synthesis methods, a change in capping agent type or concentration (i.e., stoichiometry) permits a change in NPs size. In the case of thiol-stabilized Au–NPs, this results in a diameter variation ranging from 1 to 4 nm. In the case of noble metal NPs, small seeds are typically produced by rapid reduction (e.g., HAuCl4 + NaBH4) in the presence of a stabilizing agent (e.g., sodium citrate), followed by the addition of a weak reducing agent (e.g., ascorbic acid) and a second stabilizer (e.g., CTAB). The particle growth is stimulated by repeatedly adding minute quantities of precursor. In order to prevent the formation of additional (small) particles, the addition of a "mild reducing agent" frequently slows down the reduction of the precursor. In practice, size control relies more on extensive experimental trial-and-error strategies than on a deliberate synthesis process design [20].

1.4 Nucleation Mechanism and Involved Thermodynamics

Nucleation of new particles from molecular precursors plays a crucial role in the synthesis of nanomaterials [19]. In order to create nanoparticles with the desired properties (size, shape, composition, morphology, etc.) and to accurately predict their behavior, a deeper comprehension of the fundamental physical and chemical processes governing nucleation is foremost. In homogeneous nucleation, the free energy typically increases with increasing cluster size indicating decreasing stability until the critical cluster, after which the free

energy decreases, permitting clusters to grow until the supersaturated state is attenuated [21,22]. The nucleation process necessitates overcoming a free energy barrier as a function of cluster size. However, nucleation is a dynamical process and the central quantity of interest is the nucleation rate, Therefore, it is not sufficient to simply understand the cluster thermodynamics involved in the nucleation process, but an understanding of the kinetics of cluster is needed as well. [9,23,24]

1.5 Concept of colloidal stability

One of the most important aspects in colloid science is the mechanism involve in the stabilization of NPs in the dispersing medium [20]. In general, particles at the nanoscale are unstable and tend to aggregate because at short interparticle distances due to which they are attracted toward each other by van der Waals, electrostatic or magnetic forces. Without any counteractive repulsive forces, NPs agglomerate or undergo coalescent processes. Which result in permanent grain boundary fusion and formation of larger size nanoparticles. Exemplarily, such repulsive forces can be achieved by electrostatic or steric stabilization. Electrostatically stabilized NPs are described to have at least one electrical double layer due to a surface charging. The resulting Coulombic repulsion forces between the particles decay exponentially with particle-to-particle distance. If the electrostatic repulsion is sufficiently high, it prevents the particles from any kind of coagulation. Whereas in steric stabilization, to counteract the attractive forces of Vander Waals, the steric mechanism modifies the surface of nanoparticles. Covering the surface of nanoparticles with surfactants or additives bonded to the particle surface by means of long polymer chains typically results in enhanced screening of interparticle attraction forces., According to the available literature, chemical treatment (control of pH value and addition

of surfactant) and physical treatment (ultrasonication) of colloidal solution can increase the dispersion stability of nanoscale material [17,25–27].

1.6 Size Restriction and Colloidal Stability (Kinetic and thermodynamic control)

The particles endure significant expansion as they age in suspension. One may question why thermodynamic and kinetic parameters are effective in controlling and stabilizing particle size [20]. Due to the well-known Ostwald ripening process, the majority of ultra-divided systems are thermodynamically unstable in suspension and spontaneously evolve more or less rapidly. Particle growth and/or coalescence phenomena are typically characterized by dissolution-crystallization. The propelling force of the process is the interfacial tension, which decreases the specific surface area to contribute negatively to the free enthalpy of transformation [7,28]. When the surface area of the dispersed system is at its smallest (one particle containing all the matter), true equilibrium is achieved. In actuality, because solubility is inversely proportional to particle size and because of kinetic limitations in the dissolution rate of oxides, the dissolution crystallization process ceases at a relatively advanced stage. However, one can regard particles in a dispersion to be in a state of thermodynamic equilibrium if the particle size does not change over time [19,24,29].

1.7 Application of nanomaterials in the biomedical field

The application of Nanomaterials has revolutionized the biomedical field with their unique properties and diverse applications. Here are some key areas where nanomaterials have made significant contributions.

1.7.1 Drug Delivery

Nanomaterials enable targeted and controlled drug delivery systems. Nanoparticles, such as liposomes, polymeric nanoparticles, and dendrimers can encapsulate drugs protect them from degradation and release them in a controlled manner at the desired site. This enhances drug efficacy reduces side effects and allows for personalized medicine approaches [30].

1.7.2 Imaging

Nanomaterials are widely used in various imaging techniques for enhanced visualization of tissues, cells, and molecular targets. Quantum dots, gold nanoparticles, iron oxide nanoparticles, and carbon nanotubes are utilized as contrast agents in techniques like fluorescence imaging, magnetic resonance imaging (MRI) [31], and computed tomography (CT) imaging [32]. These nanomaterials offer improved sensitivity, resolution, and multiplexing capabilities for accurate diagnosis and monitoring of diseases [33].

1.7.3 Biosensors and Diagnostics

Nanomaterials have transformed biosensing and diagnostics by enhancing sensitivity and selectivity. Nanomaterials such as nanoparticles and carbon nanotubes, with their huge surface area, allow for efficient biomolecule immobilization, enhancing detection capabilities. Their conductive nature benefits electrochemical sensors, and functionalization improves selectivity. Nanostructures, such as nanoclusters, enable accurate imaging for diagnostics. These materials, which are frequently used in point-of-care devices, provide quick and portable solutions, indicating potential advances in disease diagnosis and healthcare [34,35].

1.7.4 Theragnostic

Nanomaterials merge diagnostic and therapeutic functions into a single system known as theragnostic. They can simultaneously deliver therapeutic agents and monitor treatment response using imaging techniques. This approach allows for personalized medicine as the treatment can be adjusted based on real-time feedback from the imaging component, enhancing treatment efficacy and reducing adverse effects [36,37].

1.7.5 Bioimaging and Photothermal Therapy

Nanomaterials such as gold nanorods and carbon nanotubes have unique optical properties that can be utilized in bioimaging and photothermal therapy. They can absorb light and convert it into heat, leading to localized thermal ablation of cancer cells or targeted photothermal therapy. Furthermore, these nanomaterials can act as contrasting agents for imaging modalities like photoacoustic imaging, enhancing the visualization of diseased tissues [38].

1.7.6 Antimicrobial Applications

Nanomaterials, such as silver, copper, Zinc nanoparticles are, exhibit potent antimicrobial activity. They can be used to develop coatings for medical devices, wound dressings, and antibacterial surfaces to prevent infections and reduce bacterial resistance. These are just a few examples of the numerous applications of nanomaterials in the biomedical field. The unique properties of nanomaterials, including their small size, high surface area, tunable surface chemistry, and multifunctionality, make them promising tools for advancing diagnostics, therapeutics, tissue engineering, and disease management. Continued research and development in this area hold great potential for further breakthroughs in biomedical applications [39–41].

1.8 Common Bioimaging Techniques

1.8.1 Bioluminescence imaging

Involves the emission of light by living creatures that may or may not be genetically engineered for the aim of tracking cell localization. Thus, bioluminescence imaging does not require stimulation with a specific wavelength of external light. In bioluminescence imaging, luciferin substrate is injected into the organism's body. luciferase enzymes are either naturally occurring in the body or genetically engineered, it can interact with the luciferin substrate, resulting in light emission. Typically, the specialized camera is employed to detect emitted light. As a result, the primary advantages of bioluminescence include the absence of an external light source, which provides advantages such as minimal light scattering, the absence of ionizing radiation, high spatial resolution, accurate assessment of cell viability, and zero background noise, with the limitations of a low penetration depth (2 cm) and difficulty in genetic manipulation.

1.8.2 Fluorescence imaging

Fluorescence imaging is a technology that is utilized in many scientific disciplines, including biology and materials science. It entails the use of fluorescent molecules, which emit light when exposed to certain wavelengths of light. Researchers can construct comprehensive images of biological structures, cellular processes, and other materials by stimulating these fluorophores with a specific wavelength of light and detecting the emitted light at a different wavelength. This technology allows for non-invasive and high-resolution visualization of the spatial distribution and dynamic behavior of molecules within a sample [42–44].

Despite the fact that this imaging approach has a modest tissue penetration depth (1cm-2cm), it has various advantages such as excellent spatial resolution, high sensitivity, and uses a wide spectrum of fluorophores with unique spectral features. Furthermore, this imaging technology allows for noninvasive visualization of cells throughout the body. It is often inexpensive, using non-toxic bioimaging chemicals, and does not employ dangerous ionizing radiation. Despite the fact that the aforementioned techniques, such as CT-imaging, MRI, and SPECT, produce 3D images of the illness of interest, when compared to fluorescence imaging, they necessitate the use of toxic and expensive radioactive, contrasting, and MRI agents during cancer diagnosis. Fluorescence imaging has significant advantages over traditional CT, MRI, and SPECT imaging because it captures changes in various biological systems in real time [45]. This will allow for real-time monitoring of tumor growth, medication response, and metastatic pathways. Furthermore, with the advent of new technologies such In Vivo Live Imaging System (IVIS), soon full body fluorescence imaging system is anticipated in the market. CT, MRI, and SPECT imaging, on the other hand, require a longer acquisition time, making it impossible to catch rapid changes in tumor in real time. Fluorescence imaging, on the other hand, uses non-ionizing radiation, which protects patients from exposure during diagnostic and therapeutic operations [46]. As a result of the absence of hazardous ionizing radiation and other considerations, fluorescence imaging is a safe option for diagnosis and treatment processes in cases involving tumour growth monitoring, repetitive imaging requirements, pediatric patients, or pregnant women. Additionally, optical imaging provides high spatial resolution, allowing the visualization of fine anatomical details and cellular structures.

1.9 A brief overview of bioimaging agent/Fluorophore for optical imaging

The fluorophore is commonly used to stain tissues, cells or materials for various analytical applications. Generally, endogenous and exogeneous imaging agents are utilized for bioimaging purpose, endogenous imaging agents such as GFP (Green Fluorescent Protein) and luciferase are commonly used in bioimaging agents. GFP, which originated in jellyfish, can be coupled with target molecules to visualize cellular processes using fluorescence microscopy, revealing their kinetics and localization [47]. When paired with its substrate, luciferase, produced from fireflies and other species, permits bioluminescence, allowing researchers to monitor gene expression and biochemical activity with great sensitivity. These compounds are critical in non-invasive real-time imaging, considerably improving our understanding of complex biological phenomena [48,49]. Also, bioluminescence allows in situ light generation which helps in decreasing the inherent noise from the excitation source enabling acquisition of high signal to noise ratio. However, it is important to note that such molecules have a fixed bioluminescence intensity due to one shot biological reaction and hence in the cases where higher excitation is required such systems tend to fail to give results. Further, bioluminescent protein tends to ensue bioluminescence in the blue region, to obtain further red-shifted emission, elaborate and highly intricate molecular biological techniques and know how is required, making this whole process very complex. Also, due to their positional uncertainty, non-homogeneous scattering, autofluorescence tendency and high cost, limit their applicability in real-time. Organic dyes, quantum dots, semiconductor and metallic nanoparticles (Au, Ag, Cu) are conventional exogeneous bioimaging agents which also suffer from various limitations due to their nonbiogenic origin.

They often exhibit cytotoxicity, photobleaching, limited Stokes shift, poor photostability, non-specific binding, limited tissue penetration, limited targeting abilities, and inadequate biodegradability. These limitations hinder their use in live cell imaging, in vivo applications, long-term studies, and deep tissue imaging. Overcoming these limitations is essential to develop next-generation fluorescent nanomaterials that offer improved biocompatibility, photostability, stability in physiological conditions, tissue penetration, targeting capabilities, and biodegradability, enabling more precise and reliable bioimaging for various biomedical applications [50–52].

1.10 A brief overview of conventional bioimaging Agents and their disadvantages

1.10.1 Quantum dots

Quantum dots (QDs), are a popular choice for fluorescence imaging applications [53,54]. Their optical properties enable multiplexing, where different colors of QDs are used in a single assay with only one excitation source. QDs that emit in the NIR include II–VI, IV–VI, and III– V compounds, for example CdSe, CdTe, HgTe, PbS, PbSe, PbTe, InAs, InP, and GaAs, alloys of these component materials, and even core/shell structures, which can tune the emission further and alter fluorescence lifetimes [55,56]. An interesting probe is self-illuminating QDs, using fluorescence resonance energy transfer (FRET) from bioluminescent proteins conjugated to the QDs. However, potential toxicity from the heavy metal ions may preclude their use in clinical bioimaging and may limit their uses to in-vitro and diagnostic assays. However, they have been used successfully in a sentinel lymph node mapping procedure that utilized intraoperative NIRF imaging [57].

1.10.2 Dye-doped silica nanoparticles

NIR dye-doped silica NPs are becoming popular choices of contrast agent for a number of reasons. Silica NPs are optically transparent, water dispersible, biologically inert, nontoxic in the amorphous form, with well-established conjugation strategies to modify the surface to proteins, peptides, and other ligands for cellular receptors using silane chemistry [58] . Utilizing this matrix, numerous NIR fluorophores can be encapsulated, reducing the potential toxicity of these fluorescent probes and shielding the NIR emitter from the aqueous environment, where the dye usually suffers from low fluorescence quantum yield, degradation, and insufficient photostability. Dyes emitting in the NIR that can be incorporated into silica NPs include polymethines (e.g., Cy5.5, Cy7), indocyanine green (ICG), Alexa Fluor 750, and IRDye78, among others [59]. Encapsulating thousands of dye molecules within one silica NPs provides a tremendous advantage like a single NP loaded with dye molecules is much brighter and more stable than its single molecule counterpart. Dye-doped silica NPs are usually synthesized by a sol–gel process (i.e., Stöber process) or a microemulsion system by simply adding the dye (or a modified form of the dye) to the silica-forming solution. In addition, the surface area of these NPs can be increased by making mesoporous silica NPs, this method enables loading an additional component into the resulting pores, for example a therapeutic agent capable of photothermal ablation or a drug able to be released at the appropriate time and location [60,61].

1.10.3 Carbon nanomaterials

Carbon-based nanomaterials are also potential NIR contrast agents for *in-vivo* imaging.

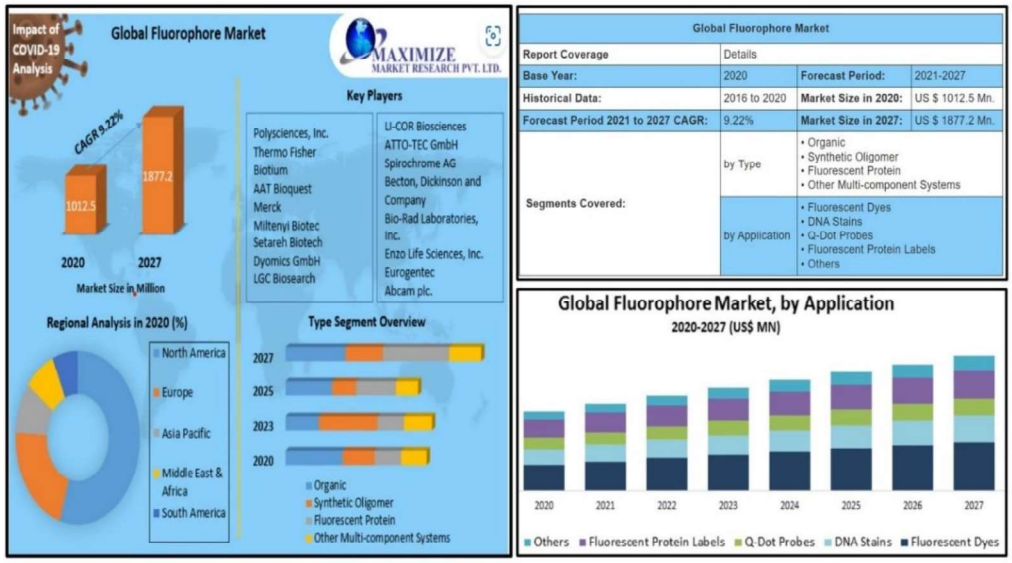
Single walled carbon nanotubes (SWNTs) can have emission in the second IR window (1000–1350 nm), which would enable even deeper light penetration [15]. However, the toxicity of SWNTs is hotly debated, and reproducible synthesis and functionalization are lacking, as are the methods to obtain high-purity samples. Carbon dots were found to have emission in the visible region when passivated by polymer chains [16]. These materials are being investigated for optical imaging agents using both one and two-photon excitation [17–19]. however, with their visible fluorescence, small animal or thin tissue imaging remains possible but clinical applications will most likely remain elusive.

Table 1.1: Drawbacks of conventional imaging agents.

1. Endogenous imaging agents	➢ Positional uncertainty ➢ Non-homogenous scattering ➢ Light penetration ➢ Expensive ➢ Need for a stable expression of Luciferase
2. Organic dye imaging agents	➢ Rapid photobleaching ➢ Not well suited for simultaneous multicolor imaging applications ➢ Susceptible to changes in a local chemical environment ➢ Emission from dyes can overlap with autofluorescence from tissues
3. Rare/transition-metal-based imaging agents	➢ Toxic in ionic form with a half-life of several weeks ➢ Deposition of free ions in bones and liver, in cases of the long residence time ➢ The high toxicity of magnetic nanoparticles restricts the use of these materials by human
4. Quantum dot imaging agents	➢ Toxic for the cell and inappropriate for any biological application ➢ The blinking property and surface of quantum dots cause deterioration of quantum dots ➢ Some of the bioconjugations of quantum dots cause difficult delivery in the target cell likely remain elusive.

Fluorophore global market size:

- Increasing demand for bright fluorescent colors in application such as biochemistry, protein study, organic light emitting diode, staining, imaging and optical brighteners is projected to boost the global fluorophore market in the next few years.
- Demand for fluorescence detection technique, which are widely used in the field of biotechnology, medical diagnostic, genetic analysis and flow cytometry is increasing.
- Market size was valued at US$ 1012.5 Mill. in 2020, and the total revenue is expected to grow at 9.22% through 2021 to 2027, reaching nearly US$ 1877.2 Mill. by 2027.
- On the flip side, the global fluorophore market is hampered by high capital spending for the development of these type of materials.



Fluorophore Market: Global Industry Analysis and Forecast

Figure 1.4. Schematic illustration of global fluorophore market [62]

1.12 Ideal bioimaging agent

Water is the main component of living organisms. Therefore, the hydrophilic NPs are extremely attractive candidates for biomedical applications such as bioimaging and antimicrobial study [26,63], Besides, the ideal NP agent must fulfill a number of stringent requirements: it should be readily dispersible and stable (i.e., resist aggregation) in a variety of polar and nonpolar solvents, hence could be utilized for number of application according to requirements [64]. Additionally, it fluorescence behavior does not affected by, different ionic strength, broad pH range, or temperature [51]. When these conditions are not intended for measurement, it should exhibit limited nonspecific binding and be resistant to the reticuloendothelial system (RES) uptake, and have programmed clearance mechanisms; and it should have high sensitivity and selectivity for the target (e.g., antigen, cell, tissue) with good contrast quality (high signal to-noise ratio, SNR) and sufficiently long circulation times in the blood if administered intravenously. Ideally, these materials will be suitable for long-term quantitative imaging at low doses and be safely cleared from the body after imaging is complete [56,65,66].

1.13 Mechanism of bacteria killing

In antibacterial activity, nanoparticles are exposed to bacterial organisms. The NPs are capable of traversing to the bacterial membrane, complementing metabolic pathways and influencing cell activity [67]. NPs then align themselves with fundamental cell components, such as DNA, ribosomes, lysosomes, enzymes and induce oxidative stress, heterogeneous changes, cell membrane permeability fluctuations, electrolyte balance abnormalities, enzyme inhibition, protein deactivation and gene expression fluctuations

[68]. Bacterial tolerance with the external environment is substantially protected by cell walls and membranes, so bacterial cell wall is essential to preserving bacterial natural structure [69]. For nanomaterial adsorption, Gram +Ve and Gram -Ve bacterial cell membrane sections employ a variety of mechanisms. Lipo-polysaccharides (LPS) are an elite Gram-negative cell wall structure and a region negatively affected by NPs. Teichoic acid is found within the cell walls of gram-positive bacteria [67,70]. NPs traverse the phosphate molecular chain and inhibit its amalgamation. Gram-positive bacteria are more effective at protecting their health if their cell walls contain LPS, lipoproteins, and phospholipids, which form a membrane that allows only macromolecules to pass through [71].

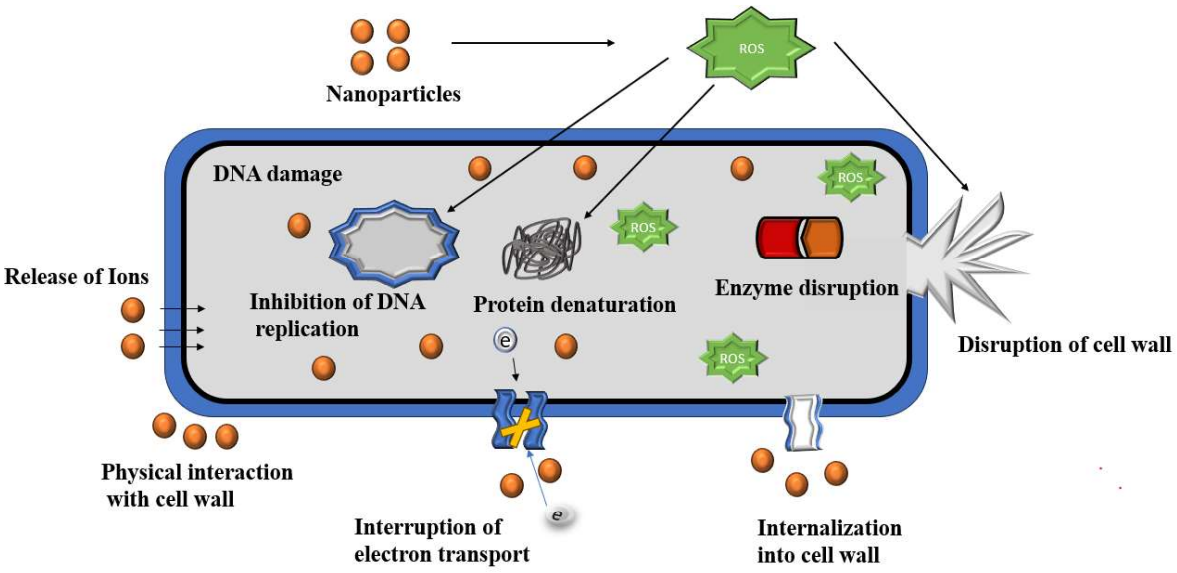


Figure 1.5: Possible pathways for bacteria cell Killing

1.14 Ideal antibacterial agent

As we are aware, Silver dominates the health care sector in terms of wound care and infection control due to their inheritant bactericidal properties, but prolonged or excessive use can lead to negative effects and potential complications [72]. Research shows that silver ions released from formulation has the potential to diffuse into the intact porcine dermis and induce DNA damage and stress response in the residing cells [73]. Magnesium is preferred as an antibacterial agent due to its biocompatibility, natural abundance, angiogenic response, catalytic response, potential for minimal resistance development, broad-spectrum antibacterial activity, synergistic effects with other treatments these properties make Magnesium as potential candidate to treat bacterial infection and wound healing [67,74].

1.15 Alkali earth metal-based nanomaterial

Alkali earth metal are present in abundance as compared to rare-earths and hence development of nanomaterials from alkali earth metals will be far cheaper provided materials with desired properties could be obtained [75]. Their (Alkali earth metals) wide availability in in the Earth's crust, makes them more readily accessible and economically viable for large-scale production. This abundance ensures a stable supply and reduces the economic barriers associated with rare materials, making alkali earth metal-based nanomaterials more practical and cost-effective for industrial applications [76]. Another crucial advantage of some of the alkali earth metals (Ca, Mg)-based nanomaterials is their excellent biocompatibility [52].

These nanomaterials are naturally occurring elements in the human body and are involved in various physiological processes [77]. Their compatibility with biological systems reduces the likelihood of adverse reactions or toxicity, making them suitable for biomedical applications. Alkali earth metal-based nanomaterials offer a safer alternative to rare earth and noble metals, which can exhibit higher toxicity or environmental hazards [74]. Their low toxicity profile enables their utilization in medical devices, drug delivery systems, tissue engineering, and other biomedical applications without compromising patient safety [3]. Alkali earth metal being II group element are highly reactive and therefore they tend to react spontaneously with other elements and water making metal hydroxides [78]. Therefore, ability to engineer these materials with have tunable properties will be of great advantage. Especially, tuning their size, shape, and surface chemistry at nanoscale will enable their customizable use to meet specific application requirements such as easy water solubility, greater colloidal stability, enhanced optical properties, which can be employed for developing solution-based biosensors, bioimaging and other optoelectronic applications [79]. Due to promising prospects, few Mg based nanomaterials were synthesized using template-based systems. Although such nanomaterial only yielded emission in blue region, which could not be used for real-time bioimaging applications. However, with the change in synthetic approach synthesis of wavelength tunable light emitting Mg-based nanomaterials are possible, which can be used in biosensing, bioimaging, photo-triggered therapies and other type of diagnostics. Also, Mg-based nanomaterials can be synthesized in such a way that they can be used for antibacterial and antifungal activity [74].

Since Alkali Earth Metal possess exceptional catalytic, biomimicking enzyme, and photocatalytic activities they can be valuable in wound healing, while alkali earth metal-based nanomaterials offer these advantages, it is crucial to consider the specific requirements and constraints of the intended application. Each class of nanomaterials possesses unique properties and characteristics that may be better suited for certain applications. In conclusion, alkali earth metal-based nanomaterials have distinct advantages over rare earth and noble metal-based counterparts. Their abundance, cost-effectiveness, biocompatibility, tunable properties, and optical and catalytic characteristics make them highly attractive for various applications such as bioimaging, nanomedicine and antimicrobial activity. Continued research and development in this area hold tremendous potential for further advancements and utilization of alkali earth metal-based nanomaterials in numerous fields.

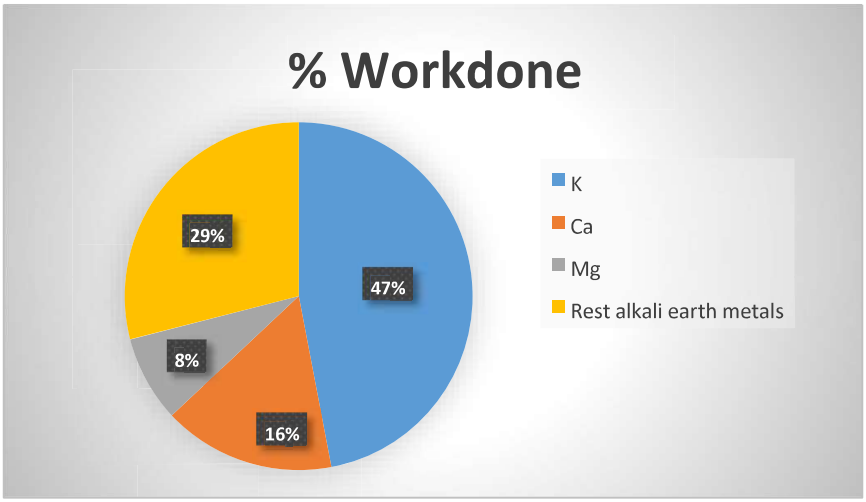


Figure 1.6: Representation of work done in alkali metal.

1.16 Magnesium-based nanomaterial in biomedical application

Magnesium-based nanomaterials have a promising future in biomedical applications. They offer unique properties such as biocompatibility, antimicrobial properties, controlled degradation, tunable fluorescence, and high surface area, making them suitable for a range of medical uses [80,81]. These nanomaterials can be employed as drug delivery systems, where they can encapsulate and release therapeutic agents at specific sites in the body [82]. Their biodegradability eliminates the need for surgical removal, particularly in the case of implants, reducing complications [83]. Nano magnesium known to exhibit antimicrobial properties along with angiogenic response, these features make them ideal candidate for wound healing. In tissue engineering, magnesium-based nanomaterials can enhance cellular processes like adhesion, proliferation and differentiation, promoting tissue regeneration [84]. They also hold potential for bioimaging and diagnostics, serving as fluorophores in techniques like fluorescence microscopy and allowing multimodal imaging with other modalities [85]. Moreover, they can be functionalized for biosensing and detection, enabling sensitive and selective analysis of biomarkers. The integration of therapy and diagnosis in theragnostic is another exciting prospect, as magnesium-based nanomaterials can deliver drugs while providing real-time imaging feedback [86]. Despite challenges related to degradation rates of magnesium, long-term biocompatibility, and controlled release, ongoing research is expected to address these issues and unlock the full potential of magnesium-based nanomaterials in biomedicine.

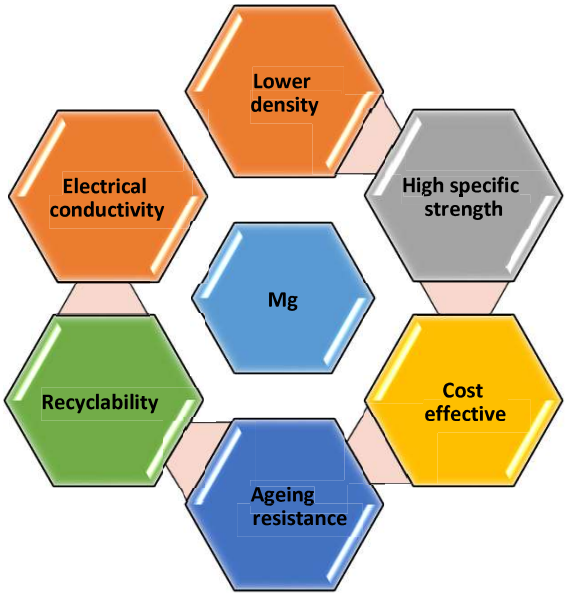


Figure 1.7. Inheritant properties of magnesium

1.17 Magnesium-based nanomaterial as fluorophore

Magnesium-based nanomaterials hold great promise as fluorophores in various biomedical applications [81]. These nanomaterials exhibit unique optical properties that make them attractive alternatives to traditional fluorophores like organic dyes or quantum dots [87].They possess excellent biocompatibility, which makes them suitable for biological systems [74]. Their low toxicity profile ensures the safety of cells and tissues during imaging studies [80]. Furthermore, magnesium-based nanomaterials demonstrate high photostability, enabling prolonged excitation without significant degradation or photobleaching [85]. This characteristic is crucial for capturing accurate and reliable imaging data over extended periods [50]. The emission wavelength of magnesium-based nanomaterials can be tuned, allowing for multiplexing in cell imaging.

By incorporating different dopants, these nanomaterials can emit light at different wavelengths simultaneously, facilitating the visualization of multiple cellular targets or processes within a single sample. Moreover, magnesium-based nanomaterials can be functionalized with targeting ligands or attached to other imaging agents, enabling multimodal imaging approaches. This integration with other imaging modalities, such as MRI or CT provides complementary information and enhances the overall imaging capabilities [31]. While challenges remain, such as improving stability and optimizing synthesis methods, ongoing research and development efforts are expected to overcome these obstacles. The future of magnesium-based nanomaterials as fluorophores in cell imaging holds tremendous potential for advancing biomedical research, diagnostics, and therapeutic applications. Their biocompatibility, tunable emission, high photostability, and potential for multimodal imaging make them valuable tools for visualizing and understanding complex cellular processes.

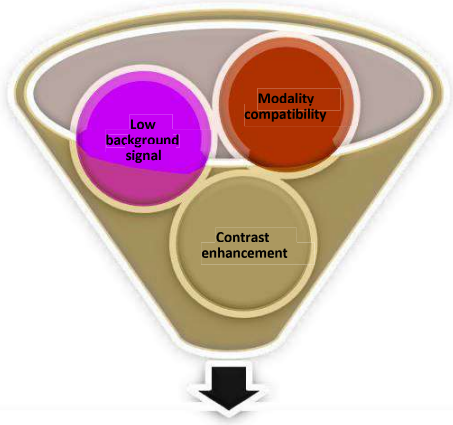


Figure 1.8. Schematic representation of Mg properties as a fluorescent probe.

1.18 Problem Statement Defined

Metallic nanomaterials have emerged as powerful tools in various biomedical applications, including bioimaging, biosensing, antimicrobial activity, drug delivery, and nanomedicine[2,81]. However, there is a critical need to develop biocompatible nanomaterials that can be safely utilized in vivo without causing adverse effects or compromising cellular functions [88]. Conventional nanomaterials often suffer from issues such as cytotoxicity, colloidal instability, limited emission wavelength, non-specific targeting, and poor biodegradability, which hinder their widespread implementation in clinical settings [3]. Among metals, magnesium is considered comparatively safer for animals and environment. The Food and drug administration (FDA) from the U.S has also recognized Mg based nanomaterial as a safe alternative [74] with exceptionally efficient properties including high optical and electrical band gap, thermodynamic stability, biocompatibility, low refractive index and dielectric constant [86,89]. Additionally, the by-product of Magnesium nanomaterials i.e., magnesium ions are completely absorbed in body without accumulating in system [3]. These distinct properties of Mg nanomaterials have resulted in inquisitiveness scientific research into the production, characterization, and application of Mg based nanomaterial. Despite having remarkable properties, synthesizing metallic magnesium nanomaterials is challenging due to their high reactivity, susceptibility to oxidation, and potential colloidal instability during fabrication owing to their lower reduction potential (-2.37 V)[90–92]. Additionally, achieving multifluorescence property in magnesium without mixing dopants and impurities is also challenging due to its limited intrinsic fluorescence properties.

It may require novel approaches such as surface modification [93], or the exploitation of unique physical phenomena to induce multiple emission wavelengths in magnesium while maintain its structural integrity. Whereas, Achieving a controlled and sustained release of Mg ions from the nanoparticles without depleting their content too quickly is requisite to impede microbial infections [70] , With this motivation, the development of magnesium-based nanomaterials that possess excellent biocompatibility, colloidal stability, tunable fluorescence, angiogenic response, antimicrobial effect and biodegradability, researchers can significantly enhance the effectiveness and safety of diagnostic tools, imaging agents, and therapeutic modalities, ultimately leading to improved patient outcomes and personalized medicine.