

## Review Article

# Green Synthesis of Silver Nanoparticles from Desert Plant Extracts from the Qassim Region for Optical Sensing Applications: A Comprehensive Review

Fahad A Alotaibi<sup>1</sup>, Fatimah I Alqannas<sup>1</sup>, Abdullah H Alluhayb<sup>1</sup>, Reham Ali<sup>1</sup> , Sayed M Saleh<sup>1</sup> <sup>1</sup>Department of Chemistry, College of Science, Qassim University, Buraidah, Saudi Arabia

## ABSTRACT

The synthesis of luminous metal nanoclusters (MNCs) from natural plant sources is a long-lasting and cost-effective approach to developing improved optical sensors. This thorough study examines the potential of two desert plant species native to the Qassim region of Saudi Arabia as biomediators for the green synthesis of silver nanoclusters (AgNCs) for optical sensing. Desert plants accumulate a wide range of secondary metabolites, such as flavonoids, phenolic compounds, and tannins, that are effective at stabilizing and reducing MNCs. Silver nanoclusters exhibit strong photoluminescence, significant Stokes shifts, and high selectivity for heavy metal ions, making them ideal for building inexpensive, easy-to-use optical sensors. This review brings together what we know about (1) the botanical and phytochemical properties of plant extracts from the Qassim region, (2) the principles and mechanisms of green synthesis of AgNCs using plant extracts, (3) the optical properties and sensing mechanisms of luminescent AgNCs, (4) recent advances in AgNC-based fluorescence sensors for finding toxic metal ions and biomolecules, and (5) the possible scale-up and practical use of plant-derived AgNCs in clinical and environmental settings. The focus is on creating optical sensors that are sensitive, selective, and reversible, with detection limits in the nanomolar range, by employing fluorescence quenching and amplification methods. The combination of traditional ethnobotanical knowledge with current analytical chemistry and nanotechnology demonstrates that it is possible to develop new optical biosensing platforms using plants native to Saudi Arabia.

**Keywords:** Silver nanoclusters, Green synthesis, Heavy metal ion detection, Qassim region, Sustainability

## INTRODUCTION

### Background: Desert flora and secondary metabolites

An area of dry and semi-dry steppe spans much of the Kingdom of Saudi Arabia, characterized by low and variable precipitation, temperature fluctuations, and vast stretches of sand, which together drive interactions within natural vegetation.<sup>[1-5]</sup> The region of Qassim within the country's central portion represents the archetypal Saharo-Arabian region, where naturally occurring flora are adversely affected by abiotic stresses such as drought, salinity, and large temperature fluctuations between day and night.<sup>[6,7]</sup> Yet even Qassim boasts a variety of xerophyte and halophyte species that provide crucial ecosystem services, such as sand stabilization, wind-erosion prevention, biodiversity facilitation, and forage and herbal resources for human use.<sup>[8-10]</sup>

From a chemical and biochemical perspective, researchers recognize wild desert plants as valuable sources of secondary

metabolites like phenolic acids, flavonoids, tannins, saponins, and alkaloids.<sup>[11,12]</sup> The primary active ingredients generally impart substantial antioxidant, antimicrobial, and anti-inflammatory activities, as well as pharmaceutical and nutraceutical value.<sup>[13,14]</sup> However, many of these secondary metabolites possess reducing and chelating abilities for the green synthesis of metal nanoparticles and nanoclusters. Therefore, secondary metabolites serve as a point of intersection among phytomedicine, analytical chemistry, and nanotechnology, thereby adding value to the naturally occurring vegetation of Qassim.<sup>[15,16]</sup>

### Desert plants of Qassim

These Qassim desert plants represent ecologically important genera with recognized chemical potential that remains underexplored in nanotechnology, particularly as green platforms for the synthesis of luminescent silver nanoclusters.

\*Corresponding author: Prof. Sayed M Saleh, Ph.D., Department of Chemistry, College of Science, Qassim university, Buraidah, 51452, Saudi Arabia. E.Saleh@qu.edu.sa

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Their long-standing use in local food and traditional medicine underscores the rich bioresource they represent. In this context, researchers have only recently begun to characterize its phytochemical and bioactivity profiles systematically.<sup>[17,18]</sup>

### ***Ecological and ethnobotanical context***

The first taxon is a slight, succulent annual characteristic of sandy substrates, typically emerging after seasonal rainfall and contributing to the floristic diversity of arid habitats in the northern, eastern, and central parts of Saudi Arabia. People living in desert communities use its seeds and aerial parts as a seasonal flavoring agent and as a supplement to staple foods such as bread or porridge-like preparations, highlighting their role as both nutritional and cultural components. The second taxon is a perennial shrub regarded as one of the most characteristic pastoral elements of desert rangelands, providing forage for camels and small ruminants and contributing significantly to dune stabilization and landscape integrity. In traditional Saudi medicine, natives use preparations from this shrub to address gastrointestinal complaints, menstrual disorders, and various dermatological conditions, reflecting accumulated empirical knowledge about its therapeutic value.<sup>[19-22]</sup>

### ***Phytochemistry and bioactivity***

Recent metabolomic investigations of the annual succulent from Qassim and surrounding regions have demonstrated considerable phytochemical diversity in the aerial parts and seeds, including phenolic constituents, flavonoids, and other secondary metabolites associated with pronounced antioxidant capacity. These findings support its classification as a promising source of natural reductants and stabilizing agents for green nanomaterial synthesis, in addition to its nutritional and traditional uses. *In vivo* and *in vitro* pharmacological studies on the perennial shrub confirm multiple bioactivities, including antioxidant effects, protection in experimental animal models, wound-healing potential, and antimicrobial action against selected pathogens. Such multifunctional profiles are beautiful when considering these extracts as bio-mediating systems in nanotechnology, where intrinsic biological activity can complement and enhance the properties of synthesized metallic nanostructures.

### ***Relevance to green nanotechnology***

Within the broader framework of sustainable nanomaterial production, these Qassim plants exemplify desert-adapted taxa that combine ecological robustness with a rich phytochemical repertoire suitable for benign synthesis routes. Their documented antioxidant and protective properties, together with traditional evidence and emerging metabolomic

data, position their extracts as strategic candidates for developing luminescent silver nanoclusters and related non-systems using environmentally friendly, plant-mediated protocols.<sup>[23-28]</sup>

### ***Metal nanoclusters in analytical chemistry***

#### ***Definition and significance of metal nanoclusters (MNCs)***

Metal nanoclusters (MNCs) are a distinct class of nanomaterials, typically consisting of fewer than 100 metal atoms, usually in the sub-nanometer to few-nanometer size range (< 3 nm).<sup>[29,30]</sup> Unlike larger metal nanoparticles (NPs) that exhibit localized surface plasmon resonance (LSPR), MNCs possess discrete electronic energy levels due to quantum confinement effects, resulting in strong size-dependent optical and electrochemical properties. The distinctive characteristics of MNCs include intense photoluminescence with significant Stokes shifts (>150 nm), excellent photostability, low toxicity, and remarkable selectivity for detecting various analytes.<sup>[31,32]</sup> Silver nanoclusters (AgNCs) are particularly attractive for analytical applications due to their exceptional photoluminescence properties, with fluorescence quantum yields reaching 10-50% and excitation/emission maxima typically in the blue-green region of the visible spectrum. The fluorescence of AgNCs is highly sensitive to environmental conditions, including pH, ionic strength, and the presence of specific analytes, making them ideal candidates for optical sensing and biosensing.<sup>[33-36]</sup>

#### ***Advantages of green synthesis of MNCs***

Green chemistry principles emphasize the design of chemical processes and products that reduce or eliminate the use and generation of hazardous substances.<sup>[37,38]</sup> Traditional synthesis methods for MNCs often employ toxic reducing agents (e.g., NaBH<sub>4</sub>, citrate), harsh conditions, and organic solvents that pose environmental and health risks. In contrast, green synthesis using plant extracts leverages naturally occurring phytochemicals as both reducing and stabilizing agents, offering numerous advantages:<sup>[39-42]</sup>

Using renewable, biodegradable plant materials can achieve environmental sustainability and eliminate toxic chemical waste. Cost-effectiveness arises from the low cost and abundant availability of plant biomass in many regions. The overall process is simple, relying on one-pot synthesis under mild conditions such as room temperature and neutral pH, without the need for toxic reagents. Inherent biocompatibility is often improved when plant-derived capping agents are present, which is advantageous for biomedical applications. The use of indigenous flora also supports local economies by promoting sustainable livelihoods in arid regions. Avoiding high-temperature or high-pressure procedures can reduce

energy consumption, thereby lowering overall energy requirements.

### ***Mechanisms of green synthesis: role of phytochemicals***

Plant extracts contain diverse phytochemicals that mediate the reduction of metal ions and subsequent stabilization of the resulting nanoclusters. The principal classes of phytochemicals involved include:<sup>[43-46]</sup>

#### *Phenolic compounds*

Including simple phenols, phenolic acids, and complex polyphenols like tannins. These compounds possess strong reducing power due to their hydroxyl groups, which can donate electrons to reduce metal ions (e.g.,  $\text{Ag}^+$  to  $\text{Ag}^0$ ) while being oxidized to quinones.

#### *Flavonoids*

A diverse class of polyphenolic compounds with antioxidant and reducing properties. Flavonoids can reduce metal ions and subsequently bind to the metal nanocluster surface via  $\pi$ - $\pi$  interactions and hydrogen bonding, thereby stabilizing the nanocluster.

#### *Terpenoids and essential oils*

Volatile compounds with unsaturated bonds that can act as reducing agents. Terpenoids may also stabilize the nanocluster by coordinating metal atoms on its surface.

#### *Proteins and amino acids*

Natural amino acids and protein-rich plant materials can serve dual roles as reducing agents and stabilizing ligands. For example, tryptophan-capped AgNCs (Tryp-AgNCs) synthesized via microwave-assisted methods exhibit intense green fluorescence and remarkable selectivity for Fe(III) ions, with detection limits as low as 16.99 nM. The amino acid scaffold provides both reduction capacity and a protective protein-like corona around the nanocluster.<sup>[47-49]</sup>

#### *Polysaccharides and sugars*

Natural reducing sugars and complex polysaccharides contribute to reducing power and may provide a hydrophilic stabilizing shell.

## **Optical properties and sensing principles of AgNCs**

### ***Photoluminescence of AgNCs***

Photoluminescence of AgNCs originates from transitions between discrete electronic energy levels created by quantum confinement of electrons to the nanocluster volume.

The characteristic features of AgNC photoluminescence include:<sup>[24]</sup>

#### *Excitation and emission wavelengths*

AgNCs typically exhibit excitation maxima in the 350-420 nm range and emission maxima in the 450-650 nm range, depending on cluster size, composition, and surface coating. For example, coffee Arabica seed extract-capped gold nanoclusters (CASE-AuNCs) display stable emission across a wide pH range and serve as dual-functional sensors for  $\text{Cu}^{2+}$  and  $\text{Hg}^{2+}$  detection.<sup>[50,51]</sup>

#### *Large Stokes shift*

The significant separation between excitation and emission wavelengths (>150 nm) minimizes background autofluorescence and enables sensitive detection without extensive optical filtering.

#### *Quantum yield*

Quantum yields for plant-capped AgNCs typically range from 1-50%, depending on the protecting ligand and synthesis method. Higher quantum yields correlate with more vigorous fluorescence intensity and improved sensitivity.

#### *Photostability*

AgNCs exhibit excellent photostability, enabling prolonged sensing applications and repeated measurements without loss of fluorescence intensity.

### ***Fluorescence quenching and enhancement mechanisms***

Changes in AgNC fluorescence intensity form the basis of most optical sensing applications, which exploit two principal mechanisms:

#### *Fluorescence quenching (Turn-Off sensing)*

Interaction of the analyte with the AgNC surface or its immediate environment leads to a decrease in fluorescence intensity. Quenching mechanisms include electron transfer from the nanocluster to the analyte, energy transfer (Förster resonance energy transfer, FRET), photoinduced electron transfer (PET), and direct interactions that lead to structural changes in the nanocluster or its protecting ligand.<sup>[52]</sup>

#### *Fluorescence enhancement (Turn-On sensing)*

A rare but valuable mechanism where interaction with the analyte increases fluorescence intensity. Enhancement can result from: the prevention of charge transfer, which usually quenches fluorescence,<sup>[53]</sup> conformational changes in the

protecting ligand that enhance emission,<sup>[54]</sup> and relief of photoinduced electron-transfer inhibition.<sup>[55]</sup>

### **Selectivity and detection limits**

The selectivity of AgNC-based sensors for specific analytes derives from:

Affinity of the protecting ligand: Different amino acids, proteins, and organic molecules exhibit varying binding affinities for different metal ions and biomolecules.

### *Size and electronic properties of the nanocluster*

Smaller clusters may exhibit enhanced selectivity due to discrete electronic levels.

pH and Environmental Conditions: Many analytes exhibit pH-dependent interactions with AgNCs.

Masking agents: Competitive chelation with masking agents can enhance selectivity by preventing quenching from interfering with ions. Recent advances in AgNC-based sensing have achieved detection limits in the nanomolar to picomolar range for various analytes. For instance, tryptophan-stabilized AgNCs (Tryp-AgNCs) for Fe(III) sensing achieve a limit of detection (LOD) of 16.99 nM, with excellent selectivity demonstrated via fluorescence quenching.

## **PHYTOCHEMICAL COMPOSITION OF QASSIM DESERT PLANTS**

These Qassim desert plants display a phytochemical richness that supports both their traditional uses and their application as green mediators for silver nanocluster synthesis. Their extracts contain high levels of phenolics, flavonoids, amino acids, and other secondary metabolites, collectively providing potent antioxidants and conferring nanotechnology-relevant properties.

### **Phytochemical composition of the annual species**

Recent phytochemical investigations on the seeds, flowers, and aerial parts of the annual taxon from Qassim and neighboring regions have revealed a diverse secondary metabolite profile with pronounced redox activity. Quantitative metabolomic studies report total phenolic contents typically in the range of 40-120 mg gallic acid equivalents per gram of dry material, depending on extraction solvent and plant organ, confirming a substantial pool of redox-active constituents. In addition to phenolics and flavonoids, detailed analyses of the floral parts indicate the presence of both essential and non-essential amino acids, supporting its nutritional value and highlighting the potential for amino acid-based scaffolding in nanocluster formation. Isolation work has also revealed constituents with antioxidant and anti-hyperlipidemic properties, many of

which possess reducing potentials compatible with metal ion reduction pathways.

### **Antioxidant and reducing behavior of the annual species**

The antioxidant behavior of extracts from this annual plant has been characterized by using several standard assays, demonstrating strong electron-donating and radical-scavenging activities. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging measurements typically yield IC<sub>50</sub> values of 15-45 µg mL<sup>-1</sup>, while 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assays confirm moderate to strong scavenging capacity, consistent with a dense phenolic/flavonoid matrix. Ferric reducing antioxidant power (FRAP) evaluations indicate substantial reducing capacity, suggesting that these extracts can donate electrons to redox processes that can convert silver ions into metallic silver within nanoclusters, thereby enabling plant-mediated synthesis of luminescent silver nanostructures without the need for harsh chemical reductants.

### **Phenolic and flavonoid composition of the perennial shrub**

The perennial shrub from the Qassim desert flora exhibits a wealthy phenolic and flavonoid profile that often rivals or exceeds that of many commonly studied medicinal plants. Studies employing Folin-Ciocalteu and colorimetric methods report total phenolic contents typically between 50-180 mg gallic acid equivalents per gram of dry extract, together with flavonoid levels in the range of 20-80 mg quercetin equivalents per gram. Within this phenolic fraction, several subclasses of phytochemicals, including phenolic acids such as caffeic and chlorogenic acids, catechin-type compounds, and more complex polyphenolic structures, are identified, which contributes to its overall antioxidant and metal-interacting properties. Such compositional complexity provides multiple functional groups that can coordinate metal ions and stabilize nascent nanostructures.

### **Comparative antioxidant and chelating properties**

Comparative evaluations of extracts from the annual and perennial Qassim taxa reveal broadly similar antioxidant potencies with some quantitative differences depending on assay conditions. DPPH radical-scavenging IC<sub>50</sub> values for both fall within 10-50 µg mL<sup>-1</sup>, with the perennial shrub occasionally showing slightly more vigorous activity in specific experimental setups. Both plants also exhibit measurable metal-chelating capacity, although their selectivity profiles toward different metal ions can differ, suggesting that they may generate nanoclusters with distinct coordination environments and surface chemistries. Such

differential chelation behavior is especially relevant for sensing applications, as it may influence the selectivity and responsiveness of AgNCs toward analytes.

### Implications for silver nanocluster synthesis

The combined phytochemical attributes of these Qassim plants create multiple parallel pathways for green synthesis of AgNCs. The co-occurrence of phenolics, flavonoids, and amino acids provides redundant reducing systems that help ensure robust, reproducible nucleation and growth of metallic nanoclusters even under mild conditions. Following reduction, many of these same biomolecules remain associated with the nanocluster surface, acting as natural capping and stabilizing ligands that help prevent aggregation and confer colloidal stability. Because these ligands are themselves bioactive, the resulting AgNCs can inherit antioxidant or antimicrobial features, potentially enhancing their performance in biomedical or environmental applications. Moreover, optical sensing platforms can exploit the intrinsic environmental sensitivity and binding preferences of plant-derived ligands, which may impart analyte selectivity, enabling fluorescence quenching or enhancement mechanisms.<sup>[56-58]</sup>

## GREEN SYNTHESIS OF AgNCs

### Synthetic approaches and methodologies

#### *Microwave-assisted synthesis*

Microwave-assisted synthesis has emerged as a particularly effective method for rapidly producing luminescent AgNCs with controlled physicochemical properties.<sup>[59]</sup> In this approach, aqueous solutions of plant extract are mixed with silver nitrate or another suitable silver salt precursor, then subjected to microwave irradiation at controlled temperatures, typically in the range of 60-100°C, for short durations of about 1-10 minutes, leading to the rapid reduction of Ag<sup>+</sup> to Ag<sup>0</sup> and subsequent nucleation of AgNCs. This strategy combines the advantages of volumetric, homogeneous microwave heating with the green, reducing, and stabilizing capabilities of plant-derived phytochemicals, resulting in the efficient formation of stable, luminescent nanoclusters. Compared with conventional heating, microwave-assisted synthesis enables synthesis in minutes rather than hours, offers improved control over nanocluster size and optical properties, enhances reproducibility between batches, and reduces both the synthesis temperature and overall energy consumption, while remaining compatible with thermolabile plant extracts that might degrade under prolonged conventional heating. Tryp-AgNCs prepared via microwave-assisted routes are a representative example, exhibiting excitation and emission maxima at 408 and 498

nm, respectively, and showing pronounced selectivity toward Fe(III) ions, with a reported limit of detection of 16.99 nM; moreover, these nanoclusters can be readily integrated into paper test strips, enabling simple, on-site monitoring of Fe(III) in environmental samples.<sup>[60]</sup>

#### *UV-assisted photochemical synthesis*

Ultraviolet irradiation provides an alternative green synthesis route for AgNCs that does not require conventional thermal heating or microwave irradiation, thereby reducing energy input and preserving thermolabile phytochemicals within the plant extract. In this methodology, an aqueous mixture of plant extract and a silver salt precursor is exposed to UV light, typically around 365 nm, for controlled irradiation times, during which photochemical reduction of Ag<sup>+</sup> to Ag<sup>0</sup> occurs and the nascent nanoclusters are stabilized by plant-derived ligands present in the medium. Nanoclusters produced under these conditions display characteristic optical signatures, including absorption bands near 420 nm and fluorescence emission around 650 nm, with core sizes commonly reported in the narrow range of approximately 1.4-1.5 nm, indicative of well-defined ultrasmall structures. Beyond their spectroscopic features, these UV-generated AgNCs exhibit notable sensing capabilities, showing high sensitivity toward heavy metal ions such as Pb<sup>2+</sup>, where fluorescence enhancement mechanisms enable selective and sensitive detection suitable for environmental monitoring and analytical applications.<sup>[61]</sup>

#### *Room temperature aqueous synthesis*

The simplest green synthesis strategies involve room temperature aqueous mixing, in which the process relies entirely on the intrinsic reducing capacity of plant-derived phytochemicals and requires no external energy source. In this approach, an aqueous plant extract is combined with an aqueous silver salt at ambient temperature, and the reduction of Ag<sup>+</sup> to Ag<sup>0</sup> proceeds spontaneously over hours to days, ultimately yielding AgNCs stabilized by the natural ligands present in the extract. Because no heating, irradiation, or specialized equipment is required, these methods demand minimal energy input, offer the highest sustainability profile, and are inherently compatible with temperature-sensitive plant constituents while exerting a very low environmental impact. Under such mild conditions, reaction kinetics are typically slower than in thermal or microwave-assisted processes. Yet, the nanoclusters formed at room temperature often display excellent colloidal stability and high reproducibility, characteristics that are advantageous for sensing and other analytical applications.<sup>[62]</sup>

## Optimization of synthesis parameters

### *Effect of extract concentration*

The concentration of plant extract plays a critical role in controlling the kinetics and outcome of green silver nanocluster synthesis, directly affecting reduction behavior, particle size, and overall yield. At higher extract concentrations, the increased availability of reducing phytochemicals accelerates the reduction of silver ions. Still, this rapid nucleation can favor the formation of larger and more polydisperse nanoclusters, which may compromise optical uniformity and sensing performance. By contrast, intermediate extract concentrations often provide a better balance between reduction rate and growth control, typically yielding smaller, more uniform nanoclusters with enhanced, more reproducible optical properties. In terms of productivity, increasing the concentration of plant extract generally increases the yield of nanocluster formation, as more reducing and stabilizing agents are available to drive nucleation and maintain colloidal stability throughout the synthesis. Nevertheless, there is usually an optimal concentration window beyond which further increases cease to improve nanocluster quality or may even degrade it due to excessive aggregation or uncontrolled growth. Most published studies indicate that extract volume fractions of 2-10% (v/v) in the total reaction mixture tend to yield favorable outcomes, offering a good compromise among reaction rate, size control, and nanocluster stability in plant-mediated systems.<sup>[63]</sup>

### *Effect of silver precursor concentration*

Silver precursor concentration is a key parameter that governs the balance among nanocluster size control, reaction rate, and optical behavior in green silver nanocluster synthesis systems. When the concentration of silver salt (typically  $\text{AgNO}_3$ ) is kept relatively low, in the approximate range of 0.5-5 mM, it results in smaller nanoclusters because the limited availability of silver ions constrains growth and favors the formation of ultrasmall, well-defined structures. Increasing the silver concentration tends to accelerate the overall reaction rate by providing a larger pool of reducible ions, but this often occurs at the expense of size uniformity, leading to broader size distributions and potential coexistence of nanoclusters with larger NPs, which in turn alter the absorption and fluorescence signatures of the system. Since the optical properties of AgNCs, including color, absorption maxima, and emission wavelengths, are strongly size-dependent, tuning the silver precursor concentration becomes an effective strategy for modulating the photophysical response of plant-mediated nanoclusters.

Both pH and temperature significantly affect the outcome of green silver nanocluster synthesis by altering reduction kinetics, ligand protonation, and colloidal stability. With respect to pH, mildly acidic to neutral conditions, typically in the range of about 4-7, are generally favorable for nanocluster formation because they maintain a balance between sufficient reducing activity and the structural integrity of plant-derived stabilizing ligands. At extreme pH values, either strongly acidic or strongly basic, can suppress reduction, unwanted side reactions can occur, or the nanoclusters may become destabilized due to changes in ligand solubility and charge, leading to aggregation or fluorescence loss. The protonation state of phenolic, carboxylic, and amino functional groups in the plant extract is pH-dependent, and this directly influences their ability to bind silver ions, donate electrons, and cap the forming clusters, so careful pH adjustment is critical for reproducible synthesis.

Temperature also plays a central role in dictating reaction kinetics and final nanocluster characteristics, with higher temperatures generally accelerating both nucleation and growth steps. Microwave-assisted protocols typically operate at about 60-100°C, exploiting rapid, homogeneous heating to produce luminescent nanoclusters with tunable properties in a matter of minutes. In contrast, room-temperature methods rely solely on the plant extract's intrinsic reducing capacity. While they offer excellent sustainability and compatibility with thermolabile phytochemicals, they require longer reaction times, often several hours to days to complete. By judiciously controlling the silver precursor concentration, pH, and temperature, it becomes possible to tailor nanocluster size, stability, and optical response for specific sensing or biomedical applications within green synthesis frameworks.

## Characterization of green-synthesized AgNCs

### *Optical characterization*

UV-visible spectroscopy is first used to monitor nanocluster formation, revealing broad absorption features characteristic of quantum-confined silver electrons, typically in the 300-450 nm range, depending on the specific cluster size and ligand environment. Changes in shape and position provide insight into nucleation, growth, and possible transitions from ultrasmall clusters to larger NPs, enabling optimization of synthesis conditions. Fluorescence spectroscopy then defines key photophysical parameters, including excitation and emission maxima, quantum yield, and fluorescence intensity. Typical AgNCs exhibit excitation bands in the 350-420 nm range and emission in the visible region, approximately 450-650 nm, often with quantum yields of about 1-50% and Stokes shifts of 100-250 nm, consistent with strong ligand-to-metal and metal-centered electronic transitions.

### **Structural and morphological characterization**

Transmission electron microscopy provides direct visualization of individual AgNCs in the green-synthesized systems. Images commonly show size distributions centered in the 1–3 nm range, predominantly spherical morphologies for solution-phase syntheses, and lattice fringes indicative of crystalline domains within the ultrasmall metallic cores. X-ray diffraction analysis confirms the crystalline nature and phase identity of the metallic component, in which AgNCs typically display diffraction peaks corresponding to face-centered cubic silver, albeit often broadened due to their small size. In addition, mass spectrometric techniques such as Matrix-Assisted Laser Desorption/Ionization–Time of Flight (MALDI-TOF) can supply molecular-weight information, enabling estimation of the exact number of silver atoms within specific cluster species and providing a bridge between structural formulae and observed optical properties.

### **Chemical characterization**

Researchers primarily use X-ray photoelectron spectroscopy to probe the surface chemistry of green-synthesized AgNCs, providing detailed information on the elemental composition and oxidation states of silver and associated heteroatoms. Analysis of the Ag 3d core levels typically confirm the presence of metallic silver in the zero-valent state, Ag<sup>0</sup>. At the same time, small shifts or additional components can indicate oxidized surface species or interactions between silver and oxygen- or nitrogen-containing ligands at the nanocluster interface. Fourier-transform infrared spectroscopy complements this by identifying the functional groups present in the plant-derived protecting ligands that cap and stabilize the nanoclusters. Characteristic bands include N–H stretching vibrations associated with amino acids or proteinaceous components, C=O stretches arising from phenolic, carboxylic acid, or amide groups, and C–O stretching modes attributable to carbohydrates and phenolic structures, collectively confirming the organic shell responsible for both reduction and stabilization in these green synthesis systems.

## **FLUORESCENCE-BASED OPTICAL SENSING WITH AgNCs**

### **Sensing mechanisms and principles**

#### ***Analyte-induced fluorescence quenching***

The most common sensing mechanism relies on the quenching of AgNC fluorescence upon interaction with the analyte:

### **Electron and energy transfer pathways**

In an electron-transfer mechanism, the analyte, often a metal ion or another electron-accepting molecule, accepts an electron from the excited silver nanocluster, thereby returning the system to the ground state and dissipating the excitation energy as heat rather than emitting light. This pathway is exceptionally efficient for hard Lewis acids such as Fe(III), Cu(II), and Hg(II), which strongly quench AgNC fluorescence by accepting electrons. When the emission band of the nanocluster overlaps with an analyte absorption band, non-radiative energy transfer, commonly known as Förster resonance energy transfer (FRET), can occur, further decreasing the observed fluorescence intensity.

#### ***Surface binding and ligand-related effects***

Surface interactions provide a complementary quenching route, in which the analyte binds directly to the protecting ligands surrounding the silver nanocluster. Such binding can induce conformational or electronic changes in the ligand shell, alter the local environment of the metal core, or facilitate new non-radiative decay pathways, all of which can reduce fluorescence intensity. Through a combination of electron transfer, energy transfer, and ligand-mediated surface interactions, analyte-induced fluorescence quenching provides a versatile, highly sensitive platform for detecting a broad range of metal ions and molecular targets using green-synthesized AgNCs.

#### ***Fluorescence enhancement mechanisms***

Some AgNC-based sensors operate in a fluorescence “turn-on” mode, where analyte binding increases rather than decreases emission intensity.<sup>[64,65]</sup> This behavior can arise when analyte ligand interactions relieve non-radiative quenching pathways or rigidify the protecting ligand shell, thereby enhancing the radiative decay of the nanocluster excited state. In other systems, controlled aggregation of AgNCs upon analyte binding results in aggregation-induced emission, in which clustered nanoclusters become significantly more emissive than their isolated counterparts.

#### ***Metal ion detection***

MNCs, particularly AgNCs and AuNCs, have emerged as powerful fluorescent probes for environmentally and biologically relevant metal ions due to their size-dependent optical properties, high surface-to-volume ratio, and ease of surface functionalization. These systems can achieve nanomolar detection limits with good selectivity, enabling both instrumental and simple visual readout formats for real-sample analysis.<sup>[66,67]</sup>

### **Iron(III) sensing**

Fe(III) is one of the most important transition metal ions to monitor in environmental and biomedical contexts, since both iron deficiency and iron overload are associated with serious health disorders. Recent work has demonstrated that Tryp-AgNCs, prepared via microwave-assisted green synthesis, serve as a highly selective and ultrasensitive fluorescent probe for Fe(III) ions.

Tryp-AgNCs for Fe(III) detection: Microwave-assisted synthesis of Tryp-AgNCs yields intensely fluorescent green-emitting nanoclusters. Tryp-AgNCs exhibit bright green photoluminescence with excitation and emission maxima at 408 and 498 nm, respectively, and operate via a fluorescence “turn-off” mechanism in which they quench luminescence upon Fe(III) binding to the tryptophan-stabilized surface. The probe offers a low limit of detection of 16.99 nM, with a typical linear response over 50-500 nM Fe(III), and shows excellent selectivity for Fe(III) against common coexisting ions such as Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Co<sup>2+</sup>, Hg<sup>2+</sup>, and Pb<sup>2+</sup>. In addition, the system has been successfully applied to the determination of Fe(III) in environmental water samples and adapted into a simple paper test-strip format for practical field use.<sup>[68,69]</sup>

### **Heavy metal ion detection (Cu<sup>2+</sup>, Hg<sup>2+</sup>, Pb<sup>2+</sup>)**

Green-synthesized MNCs have also demonstrated excellent performance in detecting toxic heavy metals, such as Cu<sup>2+</sup>, Hg<sup>2+</sup>, and Pb<sup>2+</sup>, which are of significant concern due to their persistence and bioaccumulation.

AuNCs from coffee Arabica seeds: Highly luminescent AuNCs derived from Coffee arabica seed extract (CASE-AuNCs) use a one-pot green method for preparation, in which the plant extract serves as both a reducing and stabilizing agent. These CASE-AuNCs function as dual fluorescent sensors for Cu<sup>2+</sup> and Hg<sup>2+</sup>: Fluorescence quenching of CASE-AuNCs in the presence of Cu<sup>2+</sup> results from electron or energy transfer processes combined with strong complexation between Cu<sup>2+</sup> and functional groups derived from the coffee extract on the AuNC surface. Quenching by Hg<sup>2+</sup> arises from the strong binding affinity of Hg<sup>2+</sup> to the surface ligands and the Au core, and the use of masking agents such as EDTA for Cu<sup>2+</sup> and NaBH<sub>4</sub> for Hg<sup>2+</sup> enables selective detection of each ion even in complex matrices. The system achieves nanomolar detection limits for both Cu<sup>2+</sup> and Hg<sup>2+</sup>, making it suitable for trace-level monitoring in environmental and laboratory samples. Successful analysis of these ions in water and laboratory specimens by these systems demonstrates their practical applicability.

### **Lead (Pb<sup>2+</sup>) sensing**

Poly(methacrylic acid)-stabilized AgNCs (PMAA-AgNCs), obtained via UV irradiation photochemical synthesis, exhibit a distinct fluorescence enhancement response to Pb<sup>2+</sup> in aqueous solution. Interaction with Pb<sup>2+</sup> induces a pronounced increase in fluorescence intensity, giving rise to a “turn-on” sensing response with a linear dependence of fluorescence intensity on Pb<sup>2+</sup> concentration over the relevant analytical range. The system exhibits a detection limit of about 60 nM, which is suitable for environmental monitoring of Pb<sup>2+</sup>, and this behavior is attributed to complexation between the carboxylic acid groups of PMAA and Pb<sup>2+</sup> that modulates the local environment of the AgNCs and enhances their emission.

### **Biomarker and biomolecule detection**

Beyond metal ions, AgNCs and other MNCs have been engineered as fluorescent probes for biomolecules and clinically relevant biomarkers owing to their biocompatibility and facile surface functionalization. These systems can be tailored for pH sensing, enzyme-based assays, and specific bioaffinity recognition of proteins, DNA, and other analytes.

### **pH sensing**

Fluorescent AuNCs have been used as ratiometric or intensity-based pH probes over physiologically relevant pH ranges (pH 5-9). In such systems, pH-dependent protonation and deprotonation of amino acid or other functional groups on the nanocluster surface modulate the fluorescence intensity and/or emission wavelength.<sup>[70,71]</sup>

These pH-responsive AuNCs have been applied to live-cell imaging, intracellular pH mapping, and buffer system quality control, highlighting their suitability for biochemical and biomedical sensing.

### **Glucose and other biomarkers**

MNCs, integrated with enzyme and affinity-recognition strategies, can detect glucose and other biomarkers. Glucose oxidase coupling involves immobilizing glucose oxidase on the nanocluster surface to enable indirect glucose detection, in which enzymatic oxidation of glucose produces hydrogen peroxide, which, in turn, modulates the nanoclusters' fluorescence. Bio-affinity recognition relies on the direct functionalization of nanoclusters with specific ligands, such as antibodies, aptamers, or peptide sequences, thereby enabling selective fluorescence-based detection of target proteins, nucleic acids, and other biomolecules.

### *Paper-based and point-of-care sensing*

A significant advantage of AgNC-based probes is their compatibility with simple, low-cost paper-based analytical devices, making them suitable for point-of-care and on-site environmental monitoring.

#### *Tryp-AgNC Paper Test Strips*

Tryp-AgNCs can be immobilized on paper substrates via adsorption or covalent attachment, creating portable test strips for Fe(III) sensing. Under UV illumination, Fe(III)-induced quenching of the green emission produces an apparent, visually discernible change that the naked eye can observe. At the same time, the paper-based format preserves the high sensitivity observed in bulk fluorescence assays, enabling nanomolar Fe(III) detection in the field. The resulting devices require only inexpensive materials, no sophisticated instrumentation, and minimal sample pretreatment, which makes them highly attractive for on-site testing. Detection of Fe(III) in environmental water samples using Tryp-AgNC-based paper successfully demonstrates its use. Such paper-based and point-of-care platforms are up-and-coming for ecological surveillance and clinical diagnostics in resource-limited settings where access to advanced analytical instrumentation is restricted.

## **COMPARATIVE ANALYSIS OF QASSIM PLANT EXTRACTS**

Comparative evaluation of these Qassim plant extracts as biomediators for silver nanocluster synthesis remains relatively scarce. Yet, available data indicate distinct strengths for each system in terms of reduction behavior, stabilization efficiency, and sensing performance. Together, they illustrate how differences in phytochemical architecture within desert flora leverage tuning of nanocluster formation and function in green nanotechnology.

In terms of synthesis efficiency and nanocluster yield, reports indicate that extracts from the perennial shrub typically exhibit rapid reduction kinetics and high overall conversion of silver ions, a behavior generally associated with their high phenolic content and strong intrinsic reducing capacity. By contrast, the annual species, although similarly effective, may offer advantages for generating very small, well-stabilized nanoclusters, owing to its richer content of amino acids and protein-like constituents that can serve as soft templates and capping agents during nucleation and growth. Such biomolecular scaffolding tends to favor the stabilization of ultrasmall clusters rather than larger particulate aggregates, making this extract particularly attractive for luminescent nanocluster systems.

Regarding optical properties, preliminary evidence suggests that the botanical origin of the extract can significantly influence both size distribution and photophysical characteristics of the resulting AgNCs. Systems derived from the phenolic-rich perennial shrub often exhibit broader size distributions, consistent with rapid, less controlled reductions. In contrast, nanoclusters produced from the amino acid-rich annual plant may exhibit narrower size distributions due to more gradual, regulated nucleation and growth. These structural differences translate into variations in fluorescence output, with quantum yields, Stokes shifts, and photostability strongly depending on the nature of the plant-derived ligands bound to the nanocluster surface, which modulate its electronic environment. In some cases, the more robust ligand shells derived from complex phytochemical mixtures can provide enhanced photobleaching resistance, which is beneficial for sensing and imaging applications.

With respect to selectivity and sensing performance, interspecies differences in phenolic and related secondary metabolites generate distinct metal-binding and analyte-recognition profiles at the nanocluster interface. Phenolic subclasses bearing catechol or ortho-dihydroxy groups tend to exhibit high affinity toward trivalent and specific divalent metal ions such as Fe(III), Al(III), and Cu(II). In contrast, simpler phenolic structures may interact in a broader but less selective manner because these functional groups constitute the outer ligand shell of the nanocluster. The specific phytochemical signature of each Qassim plant extract can yield distinct binding preferences, fluorescence-quenching behaviors, and response patterns toward target analytes, thereby imparting plant-derived selectivity to the resulting silver nanocluster sensors.

## **ADVANCED ANALYTICAL TECHNIQUES FOR CHARACTERIZATION**

Advanced analytical techniques play a central role in elucidating the structural, optical, and electrochemical properties of green-synthesized AgNCs and in clarifying their sensing mechanisms. Spectroscopic and electrochemical methods together provide a multidimensional picture that links nanocluster structure to function in practical sensor architectures.<sup>[72-75]</sup>

### **Spectroscopic methods**

Surface-enhanced Raman scattering exploits the strong local electromagnetic fields generated in the vicinity of MNCs to amplify Raman signals from nearby molecules, often by enhancement factors of  $10^4$ - $10^6$ , depending on cluster size, morphology, and aggregation state. Silver and gold nanoclusters, therefore, serve as powerful SERS-active

substrates, enabling trace detection of organic molecules and environmental contaminants while simultaneously yielding rich molecular structural information through characteristic vibrational fingerprints that can support multiplexed analyte identification. Time-resolved fluorescence measurements provide complementary insight into the excited-state dynamics and sensing mechanisms of AgNCs. Typical fluorescence lifetimes for these MNCs fall in the approximate range of 10-100 ns, with values governed by core size, ligand composition, and local environment, and time-resolved data are particularly informative for distinguishing static quenching, where ground-state complexes lead to non-exponential decay, from dynamic quenching, where collisional processes in the excited state produce predominantly exponential decay behavior. Such analyses help disentangle overlapping quenching pathways and refine mechanistic models for analyte-induced fluorescence changes in sensing applications.<sup>[76-81]</sup>

### Electrochemical characterization

Electrochemical methods, especially cyclic voltammetry, complement optical techniques by probing the redox properties of AgNCs and their interactions with analytes at electrode interfaces. Size-dependent redox potentials of nanoclusters can be determined from cyclic voltammograms, providing information on their ease of oxidation or reduction and on electron-transfer kinetics, which are crucial for designing systems that couple optical readout with redox-active sensing pathways. Building on this knowledge, electrochemical sensors incorporating AgNCs as electrode modifiers or redox mediators have been developed for detecting diverse analytes, leveraging the high conductivity, catalytic activity, and rich redox behavior of nanocluster-modified surfaces to improve sensitivity and lower detection limits relative to bare electrodes.<sup>[82-87]</sup>

## ANTIMICROBIAL AND BIOLOGICAL PROPERTIES

AgNCs and the plant extracts used in their green synthesis provide a dual platform that combines antimicrobial and antioxidant functionalities with optical sensing capabilities. This multifunctionality is particularly attractive for environmental and biomedical applications where simultaneous detection and mitigation of harmful species are desirable.<sup>[88-90]</sup>

### Antimicrobial mechanisms and efficacy

AgNCs exhibit broad-spectrum antimicrobial activity via multiple mechanisms that target key cellular structures and processes in microorganisms. Controlled dissolution of silver from the nanocluster surface releases Ag<sup>+</sup> ions, which

disrupt bacterial cell wall and membrane integrity, increase permeability, and ultimately compromise cell viability, while concurrently generated reactive oxygen species under aerobic or illuminated conditions cause oxidative damage to lipids, proteins, and nucleic acids. In parallel, silver species and reactive oxygen species (ROS) can denature essential enzymes and structural proteins and interact directly with microbial DNA, inhibiting replication and transcription and driving cells toward irreversible damage and death. Experimental studies on silver nanomaterials, including NPs and preliminary nanocluster systems derived from the same plant sources, have demonstrated activity against clinically relevant bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*, as well as pathogenic fungi including *Candida albicans*, *Aspergillus flavus*, and *Trichoderma viride*. The observed antimicrobial efficacy arises from the combined action of the metallic silver core and the bioactive plant-derived ligands that cap the nanoclusters, with the latter often contributing intrinsic antibacterial or antifungal effects that synergize with the silver-based mechanisms.<sup>[91-93]</sup>

### Antioxidant properties and biomedical relevance

The protecting ligands surrounding plant-mediated AgNCs are typically rich in phenolic compounds and flavonoids, which endow the nanocluster conjugates with pronounced antioxidant activity in addition to their optical and antimicrobial functions. These phytochemicals can directly scavenge free radicals by donating electrons or hydrogen atoms, chelate transition metal ions that would otherwise catalyze radical formation via Fenton-type reactions, and, in some biological contexts, may contribute to the upregulation of endogenous antioxidant enzymes, thereby reinforcing cellular defense systems. As a result, plant-stabilized AgNCs have the potential to offer combined benefits in biomedical settings, acting simultaneously as fluorescent probes for sensing or imaging and as protective agents that counter oxidative stress associated with inflammation, infection, or environmental insults. This convergence of sensing, antimicrobial, and antioxidant properties positions green-synthesized AgNCs as promising candidates for next-generation theranostic platforms, wound dressings, and antimicrobial coatings, where both pathogen detection and reactive species mitigation are required.<sup>[94-96]</sup>

## ENVIRONMENTAL APPLICATIONS AND SUSTAINABILITY

### Environmental monitoring and water quality

In water quality assessment, metal ion contamination and excess nutrients or organic pollutants pose major environmental and public health concerns that require

sensitive, selective analytical tools. Green-synthesized AgNCs can be tailored to detect heavy metals such as Fe(III), Cu<sup>2+</sup>, Hg<sup>2+</sup>, Pb<sup>2+</sup>, As(III/V), and Cr(VI), as well as nutrient species like phosphate and nitrate, and specific organic contaminants including pesticides and pharmaceuticals, by exploiting specific fluorescence quenching or enhancement responses. A key advantage of these systems is their operational simplicity, enabling either visual readout or straightforward fluorescence measurement without the need for complex, high-end instrumentation, while still achieving nanomolar detection limits that are compatible with regulatory guidelines and offering good specificity, cost-effectiveness, and sustainability due to inexpensive reagents, simple equipment, and biodegradable components.<sup>[97,98]</sup> Paper-based silver nanocluster sensors enable point-of-care environmental testing, which allows distributed monitoring at or near the sampling site without reliance on centralized laboratories. These platforms often require minimal or no sample pretreatment, allowing direct analysis of water samples and providing semiquantitative or fully quantitative information through color or fluorescence intensity comparison by eye or via portable fluorescence readers, making them suitable for routine field surveys and rapid screening campaigns.

### Sustainability and global goals

The green synthesis approach for AgNCs aligns closely with broader sustainability frameworks and international development priorities. Their use in water quality monitoring directly supports Sustainable Development Goal (SDG) 6, Clean Water and Sanitation, by enabling optical detection of contaminants that threaten safe drinking water supplies. By relying on renewable plant materials and producing mainly benign, biodegradable byproducts, these methods advance SDG 12, Responsible Consumption and Production. At the same time, eliminating toxic reagents and reducing energy-intensive steps contribute to SDG 13, Climate Action, by lowering carbon and pollution footprints. Furthermore, when desert or underutilized plants are used sustainably, the approach can reinforce SDG 15, Life on Land, by promoting biodiversity conservation and providing income-generating opportunities in arid regions. From a circular economic perspective, the green synthesis of AgNCs from desert plant biomass exemplifies resource-efficient, low-waste manufacturing principles. The process leverages abundant, renewable plant resources instead of relying on energy-intensive, petrochemical-based synthesis routes. It typically operates in aqueous media without toxic organic solvents or hazardous byproducts, thereby minimizing waste generation and environmental burden. Plant-derived protecting ligands are inherently biodegradable, thereby reducing the long-term environmental persistence of organic components.

At the same time, the value-added utilization of locally available flora can support rural economies by creating new supply chains based on sustainable harvesting, processing, and nanomaterial production that integrate ecological stewardship with economic development.<sup>[99,100]</sup>

### CHALLENGES AND FUTURE PERSPECTIVES

Green synthesis of AgNCs has advanced considerably, yet several essential challenges related to standardization, stability, selectivity, and scale-up still limit widespread implementation.<sup>[101-103]</sup> Addressing these issues is essential to translate laboratory protocols into robust, industrially and clinically relevant technologies while preserving the sustainability advantages of plant-based approaches and ensuring that green routes genuinely outperform conventional, chemically intensive methods in real-world settings.<sup>[104]</sup>

#### Standardization, stability, and selectivity challenges

Standardization and reproducibility remain major obstacles because plant-mediated systems inherently suffer from variability in phytochemical composition driven by seasonal, environmental, geographic, and processing differences, such as harvesting time, drying procedures, and storage conditions of the biomass. Extraction conditions, including solvent polarity, solid-to-liquid ratio, temperature, and duration, further modulate the profile and concentration of active phytochemicals, and in the absence of universally accepted reference standards and validated analytical protocols for normalizing these parameters, batch-to-batch differences in nanocluster size, fluorescence intensity, and stability are inevitable, becoming even more pronounced when processes are transferred from small laboratory volumes to larger preparative or pilot-scale reactors. Nanocluster stability and storage pose additional difficulties, since ultrasmall silver cores possess high surface energy and can undergo oxidation, dissolution, or Ostwald ripening, particularly when protecting ligands are partially lost or degraded during handling and long-term storage. As a result, fluorescence intensity may gradually diminish due to photobleaching under repeated illumination, thermal degradation at elevated temperatures, or quenching by dissolved oxygen and other reactive species, thereby constraining practical shelf life and complicating distribution and field use. Most current formulations require storage as aqueous dispersions at low temperature (typically around 4 °C) and still show stability on the order of weeks to a few months; this situation highlights the need for more robust stabilizing matrices, such as polymer encapsulation, silica or biopolymer shells, and dry-state formulations obtained by lyophilization or spray drying with appropriate cryo- and lyoprotectants. Selectivity and interference

management are equally critical challenges, particularly in complex environments, such as food or biological samples, where multiple ions and organic components coexist and can simultaneously modulate nanocluster fluorescence. Multi-ion interference can lead to non-specific quenching or enhancement, obscuring the signal from the target analyte. At the same time, matrix factors such as pH, ionic strength, natural organic matter, and proteins can alter nanocluster aggregation state, surface charge, and local microenvironment, thereby affecting both sensitivity and apparent selectivity. To overcome these issues, improved strategies are needed, including ligand engineering to introduce recognition motifs and the use of masking agents that selectively bind and suppress common interferents. These sensor arrays capture multidimensional response patterns across multiple analytes, and chemometric or machine-learning algorithms can deconvolute overlapping fluorescence responses to obtain quantitative analyte information.

#### Future directions in synthesis and sensor development

Future research on synthesis optimization should prioritize high-throughput and statistically designed screening frameworks that systematically explore the multidimensional parameter space of plant extract concentration, silver precursor level, pH, temperature, reaction time, and irradiation mode to identify robust operating windows with minimal sensitivity to small fluctuations. Within this context, rational ligand design—where plant-derived ligands are selectively derivatized, fractionated, or combined with well-defined synthetic molecules, offers an attractive pathway to tune surface charge, hydrophilicity, and binding affinity while preserving core aspects of sustainability, especially in hybrid systems that couple the green reducing capacity of natural extracts with the precision and predictability of engineered chelators or polymers.

On the sensing side, next-generation AgNC-based platforms will likely emphasize multiplexing capabilities for simultaneous detection of several analytes via spectral demultiplexing, ratiometric readouts, or microarray-style sensor formats that exploit slightly different nanocluster compositions or ligand shells to encode distinct response fingerprints. Another priority is the development of reversible and regenerable probes that can bind and release analytes without significant degradation, thereby enabling multiple measurement cycles and greatly reducing the cost per analysis; such systems may rely on reversible coordination chemistry, competitive binding strategies, or stimulus-responsive ligands that can be reset by pH or ionic-strength changes. Integration with compact, portable optical readers such as smartphone-based fluorescence detectors, low-cost LED-photodiode modules,

or fiber-optic probes will be essential for field deployment in environmental monitoring, food safety, and on-site industrial quality control. At the same time, translation to biomedical applications will require rigorous analytical validation, biocompatibility assessment, and compliance with regulatory frameworks governing diagnostic devices and injectable or implantable materials.

#### Scale-up, industrialization, and fundamental studies

At the industrial level, realizing the full potential of green-synthesized AgNCs will depend on developing automated, digitally controlled synthesis systems that maintain precise control over mixing, temperature, irradiation, and reaction time, thereby minimizing human-induced variability and ensuring reproducible product quality at higher throughputs. Implementation of robust, rapid quality-control protocols that combine spectroscopic fingerprints, particle-size analysis, and perhaps simple colorimetric tests will be necessary to monitor key attributes, such as nanocluster size distribution, fluorescence intensity, and stability, for every batch. In contrast, life-cycle assessment and detailed techno-economic analysis will help benchmark these processes against conventional routes and justify investment from industrial stakeholders. Engagement with regulatory agencies at early stages will also be important for defining acceptable specifications, safety testing requirements, and labeling standards for nanocluster-based sensors and devices intended for environmental, food, or clinical markets.

Fundamental studies remain indispensable for deepening the understanding of silver nanocluster behavior and guiding the rational design of improved systems. Mechanistic investigations that integrate time-resolved spectroscopy, single-particle methods, and theoretical modeling can elucidate the precise pathways of fluorescence quenching and enhancement, including the roles of electron transfer, energy transfer, and structural reorganization within the ligand shell. Detailed structure–function studies that determine exact silver atom composition, three-dimensional arrangements of atoms, and the spatial distribution of ligands around the metal core will enable direct correlation between specific structural motifs and observed optical or sensing properties, potentially allowing predictive design of clusters with tailored emission color, quantum yield, and analyte responsiveness. In parallel, systematic studies of biointeractions, covering cellular uptake, subcellular localization, immunological responses, and *in vivo* distribution, are necessary to ensure safe and effective biomedical use. At the same time, environmental fate assessments examining persistence, biodegradation pathways, and ecotoxicity in soil and aquatic systems will help ensure that green synthesis of AgNCs translates into truly

sustainable and responsible nanotechnology throughout their entire life cycle.

A rigorous assessment of greenness is essential to objectively evaluate plant-mediated synthesis of AgNCs and their analytical applications, beyond qualitative descriptions of “eco-friendly” or “sustainable” methods. Recent green analytical chemistry metrics such as AGREE (Analytical GREENness), MoGAPI and ComplexMoGAPI, AGSA (Analytical Green Star Area), and CaFRI (Carbon Footprint Reduction Index) provide quantitative or semi-quantitative scores and pictograms that integrate multiple criteria, including reagent toxicity, solvent volume, energy consumption, waste generation, sample preparation, and analytical throughput. In the context of desert-plant-derived AgNCs, these tools could be used to compare different green synthesis routes (microwave-assisted, UV-assisted, room-temperature) and sensing formats (solution vs. paper-based), thereby enabling ranking of procedures based on their environmental impact and operational performance. A schematic figure summarizing the main features and output of AGREE, MoGAPI/ComplexMoGAPI, AGSA, and CaFRI could visually guide readers in selecting or designing AgNC-based methods that are not only analytically robust but also demonstrably greener.

## CONCLUSION

The green synthesis of luminescent AgNCs from desert plants endemic to the Qassim region represents a promising frontier in sustainable analytical chemistry and environmental sensing. The rich phytochemical composition of these plants provides natural reducing and stabilizing agents for producing optical biosensors with remarkable selectivity and sensitivity for detecting toxic metal ions and biomolecules.

Recent advances in AgNC-based sensing, particularly the development of tryp-AgNCs with a detection limit of 16.99 nM for Fe(III) and AuNCs derived from coffee seeds with dual functionality for Cu<sup>2+</sup> and Hg<sup>2+</sup> detection, demonstrate the maturity of this technology. The ability to translate these sensors into simple paper-based formats for point-of-care applications offers promise for resource-limited settings and distributed environmental monitoring. However, significant challenges remain in standardizing synthesis procedures, achieving consistent nanocluster properties across batches, ensuring long-term stability, and translating laboratory successes to industrial-scale production. Future research must address these challenges by systematically optimizing synthesis parameters, rationally designing protecting ligands, developing advanced characterization methods, and rigorously validating in real-world applications.

The convergence of traditional ethnobotanical knowledge with modern nanotechnology and analytical chemistry creates unique opportunities for innovation. By sustainably utilizing native Saudi Arabian flora, this research direction supports multiple United Nations Sustainable Development Goals while contributing valuable scientific insights into metal nanocluster chemistry and sensing mechanisms. The potential for AgNCs synthesized from Qassim desert plants extends beyond optical sensing to include antimicrobial coatings, antioxidant-enhanced biomedical devices, and catalytic applications. Continued investment in fundamental and applied research will unlock the full potential of these green-synthesized nanomaterials for addressing global challenges in water quality monitoring, disease diagnosis, and environmental protection.

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