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REVIEW PAPER

# Cow Urine Mediated Green Synthesis of Nanomaterial and Their Applications: A State-of-the-art Review

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**ABSTRACT**

Nowadays, green syntheses have received crucial attention as a reliable, developing, and eco-benevolent protocol for synthesizing a broad range of nanomaterials (NMs) including metal/metal oxides NMs, bio-inspired materials, and hybrid/composite NMs. As such, biogenic synthesis is regarded as a significant tool to mitigate the destructive impacts associated with the conventional approaches of synthesis for NMs generally utilized in industry and laboratory. In this review, we summed up the general protocols and mechanisms of green synthesis routes, especially for silver (Ag), silver oxide ($Ag_2O$), cadmium (Cd), copper (Cu), copper ferrite ($CuFe_2O_4$), palladium (Pd), aceprophyline, cellulose and graphene nanomaterials/nanoparticles using cow urine. Importantly, we explored the main role of biological constituents which has existed in cow urine. These essential biomolecules act as reducing/stabilizing agents in solvent systems. The stability, phase formation, and surface morphology of NMs using characterization techniques are also discussed. Finally, we covered the eclectic applications of such synthesized NMs in terms of anti-asthma, antimicrobial, antituberculosis, antioxidant, anticancer activity, catalytic activity, and removal of pollutants dyes.

**Keywords:** *Cow urine, Nanotechnology, Green synthesis, Applications*

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## INTRODUCTION

In Indian culture, cows are believed to be holy, venerable, and called "KAMADHENU" which means the mother of all spiritual entities since ancient times. In Hinduism, peoples use panchagavya (urine, ghee, milk, curd, and dung), which is obtained from cows and is advantageous in diverse ways as a food supplement, spiritual uses, and medicines due to its importance in mythological, spiritual, and medicinal aspects [1]. As per this reference, the cutting-edge science

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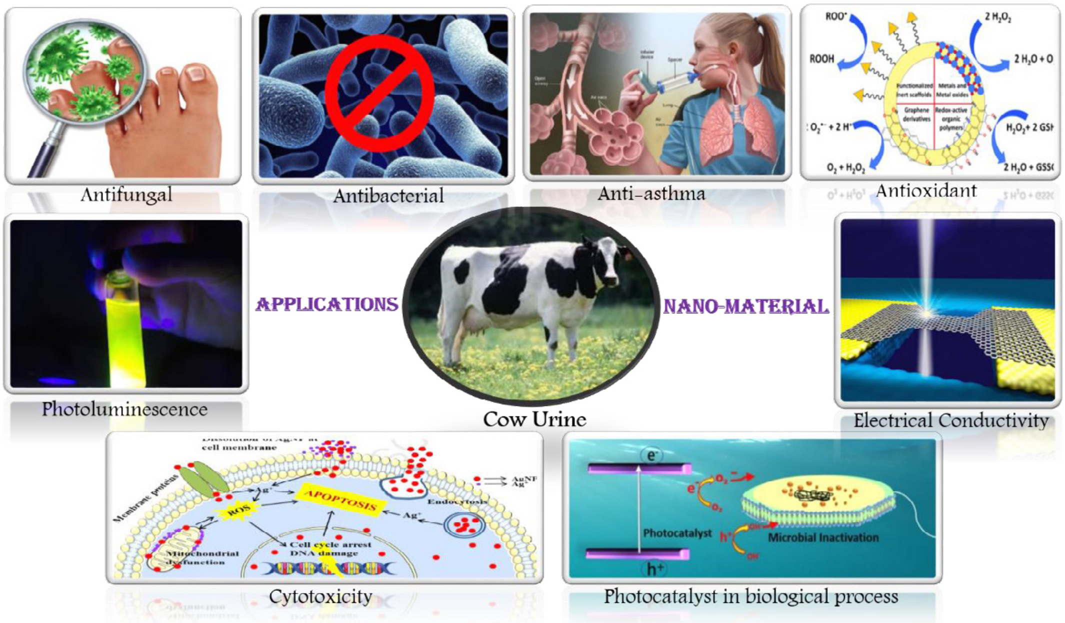


Fig. 1. Applications of NMs synthesized from cow urine

investigated cow urine and found out that it is capable of curing joint pain, blood pressure, diabetes, cardiac disease, cancer, thyroid, asthma, psoriasis, skin inflammation, headache, ulcer, gynecological issues, etc. [2]. Moreover, cow urine has good medicinal potential for the discipline of antimicrobial, antioxidant, anti-anthelmintic, anticancer, biosensors against play an important role in biotechnology. Therefore, cow urine can cure various sorts of diseases and is confirmed as an efficient as well as a potent medicinal candidate [3-17].

Recently, nanotechnology, rapidly developing due to its interesting and versatile applications in the discipline of biomedicine, bio-imaging, biosensor, optics, semiconductors, solar cells, catalysis, electrochemistry, ceramics, fuels, biotechnology, agribusiness, pharmaceuticals, textile industry, and water treatment [18-26]. In this perspective, cow urine mediated NMs in restorative recorded to give a thought of the green synthesis of NMs utilizing cow urine to progress the biomedical properties of NMs appeared in **Fig. 1**. Here, we covered the current advancement of research on the green synthesis of NMs with their advantages over chemical synthesis routes. The main aim of this review is to provide detailed protocols for green synthesis NMs using cow urine and their versatile applications.

## SCOPE OF THE REVIEW

The scope of the present review is to provide an up-to-date report on cow urine mediated NMs and their potentially versatile applications for anti-asthma, antimicrobial, antioxidant, anticancer, and photocatalytic activities, *etc*. Moreover, the structural properties of the NMs, especially the stability, morphology, size, chemical, and physical properties were discussed. Notwithstanding, representative protocols, including basic general methodologies for cow urine mediated NMs, was briefly investigated. Also, characterization techniques for NMs were described only briefly.

## BIOGENIC SYNTHESIS OF NMs

To date, innumerable synthetic approaches (**Fig. 2**) were explored for the synthesis of NMs. Therein, top-down and bottom-up are two main approaches by which scientists develop NMs having intriguing and differing utility. In a top-down approach, metal decreases up to the nano level while in bottom-up approaches NMs generates from molecules or atoms. Therefore, NMs could be easily produced by utilizing known chemical and physical strategies like co-precipitation, sol-gel, sonochemical, laser ablation, hydration techniques, hydrothermal reaction, solution combustion, reflux method, polyol, solvothermal, chemical lessening, ion-exchange, chemical vapor decomposition,

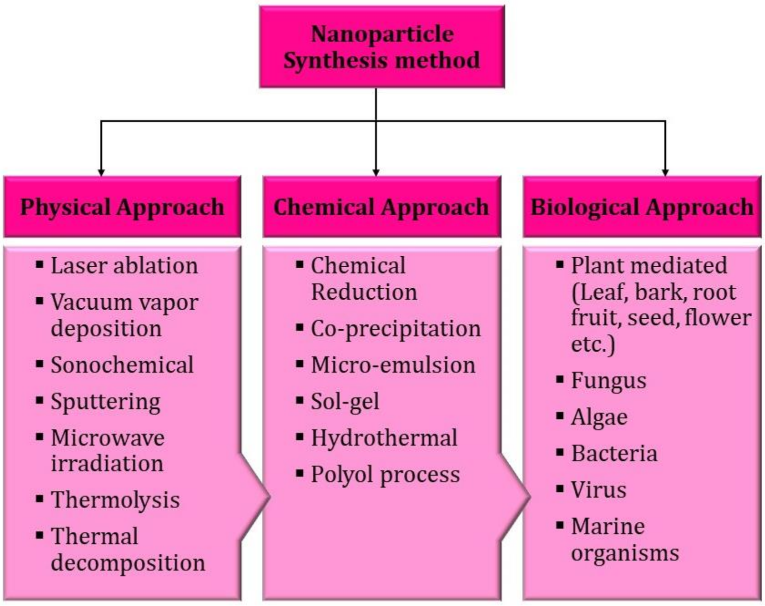


Fig. 2. Diverse synthetic approaches involved in the production of nanomaterials


Fig. 3. Benefits of green synthesis in nanoscience

colloidal method, spray pyrolysis, mechanical processing, physical vapor deposition, sputtering, laser pyrolysis, microemulsion, photochemical, electrochemical, and microwave [27-41]. However, biogenic strategies lead to synthesized NMs over known chemical and physical strategies due to their immense benefits (**Fig. 3**). Most of the chemical and physical strategies include the utilization of costly and toxic additives/solvents, highly toxic reducing and stabilizing agents, which can cause a noxious effect on both environment and human life. In contrast, biogenic synthesis of NMs is a one-pot, swift, safer, and biocompatible or eco-benevolent bio-reduction technique that requires relatively minimum energy to start the reaction.

In particular, cow urine is acknowledged around the world as a pharmaceutical over close around all kinds of disease, the hormones, vitamins, and acids

Table 1. Examples of cow urine mediated NMs

| S. No. | NMs | Method | Precursor | Characterization | Shape | Size | Reference |
|---|---|---|---|---|---|---|---|
| 1 | Aceprophyline | Solvent evaporation | Aceprophyline | FTIR, UV-vis, SEM, EDX, TEM, DSC and Zeta potential | Round and Spherical (SEM) | - | 42 |
| 2 | Ag NPs | Biosynthesis | $AgNO_3$ | XRD, FTIR, UV-vis, SEM, EDAX, PL, BET, TGA, PSA and TEM. | Agglomeration (SEM) | 11 nm (DLS) | 43 |
| 3 | Ag NPs | Hydrothermal | $AgNO_3$ | FTIR, UV-vis, SEM, EDAX, STM. | -- | 50 nm (STM) | 44 |
| 4 | Ag NPs | Biosynthesis | $AgNO_3$ | FTIR, UV-vis, SEM-EDX, and HR-TEM. | Spherical (TEM) | 20-100 nm (TEM) | 45 |
| 5 | Cu NPs | Biosynthesis | $CuSO_4$ | XRD, FTIR, UV-vis, SEM, EDAX, Laser Raman, DLS XRF, and HR-TEM. | Spherical (SEM) | 98 nm (DLS) | 46 |
| 6 | $Ag_2O$ NPs | Combustion | $AgNO_3$ | XRD, FTIR, UV-vis, SEM, EDAX, PL and TEM. | Spherical (HR-TEM) | 11 nm (XRD) | 47 |
| 7 | Cd NPs | | $CdSO_4$ | FTIR, UV-vis, FE-SEM, XRD, ZP, DLS. | Cylindrical, Poly-dispersed (SEM) | 62.2 nm (DLS) | 48 |
| 8 | Cellulose NPs | Microemulsion method | Cellulose | FTIR, UV-vis, FE-SEM, TEM, AFM EDX, XRD, and XPS. | Spherical (FE-SEM) | 30-40 nm (TEM) | 49 |
| 9 | $CuFe_2O_4$ NPs | Sol-gel auto Combustion | $Cu(NO_3)_2.6H_2O$, $Fe(NO_3)_3.9H_2O$ | FTIR, UV-vis, FE-SEM, EDX, XRD. | Spherical (FE-SEM) | 14.5-22.3 nm (FE-SEM) | 50 |
| 10 | Graphene Nanosheets | Spray Pyrolysis | Graphene Oxide | XRD, FTIR, UV-vis, XPS, FE-SEM, EDAX, BET, AFM, and HR-TEM. | Nanosheets (FE-SEM) | Length- 8 nm (Raman and XRD) Thickness - 3 nm (AFM) | 51 |
| 11 | Graphene Nanosheets | Biosynthesis | Graphene Oxide | XRD, FTIR, UV-vis, XPS, FE-SEM, EDAX, BET, AFM, and HR-TEM. | Nanosheets (FE-SEM) | - | 52 |
| 12 | Graphene Nanosheets | Solvothermal | Graphene Oxide | FTIR, UV-vis, FE-SEM, Raman and XPS. | Nanosheets wrinkled structure (FE-SEM) | - | 53 |
| 13 | Pd NPs | Biosynthesis | $PdCl_2$ | FTIR, UV-vis, FE-SEM, XRD, DLS, and Zeta potential. | Cylindricaland poly-disperse (FE-SEM) | - | 54 |

found in cow urine bio-reduce the metal salt into NMs. **Table 1** summarized the green synthesis of distinctive NMs from cow urine.

## PROTOCOL FOR BIOGENIC SYNTHESIS OF NMs FROM COW URINE

The required properties of synthesized NMs depend on their size and morphology. Therefore, NMs are synthesized by altering a few parameters such as pH, temperature, response time, and concentrations of the metal salt and cow urine. The cow urine is easily mixed with the different concentrations of selected metal salt solutions to room temperature and their conversion to respective NMs takes place within minutes inefficient, one-pot, sustainable, and eco-accommodating approach (**Fig. 4**). Due to the ample number of constituents (copper, iron, nitrogen, manganese, sulfur, carbolic acid, chlorine, silicon, magnesium, calcium salts, citric, creatinine, enzymes, mineral salts, hormones, vitamins like A, B, C, D, E, gold acids, uric acid, etc.) present in the cow urine and which act as bio-reducing and/or stabilizing agents, there is no need to add external noxious stabilizing/capping additives. The detailed protocols of biogenic synthesis of NMs by cow urine are also described by authors [36]. Finally, the NMs could be separated by centrifuging at high speed and placing in a preheated muffle furnace using ceramic crucible and calcined at the required high temperature [47]. A fine powder of NMs is obtained and collected for further application purposes.

## MECHANISM FOR GREEN SYNTHESIS OF NMs *VIA* COW URINE

Green syntheses of NMs are based on bio-reduction of metal up to nano level and cow urine is capable of producing such NMs. The chemical composition affirms the nearness of creatinine, uric acid, and leucocyte in cow urine having amide and amine utilitarian gather. In the green synthesis of NMs, commonly metal particle comes in contact

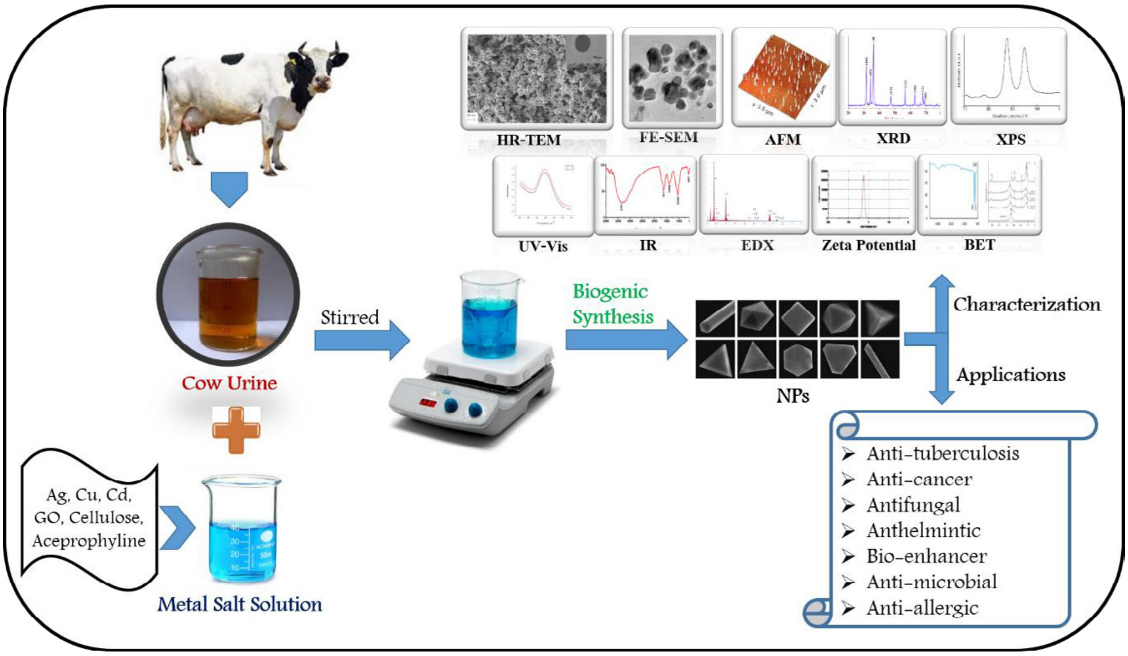


Fig. 4. Biogenic synthesis of NMs from cow urine and their uses

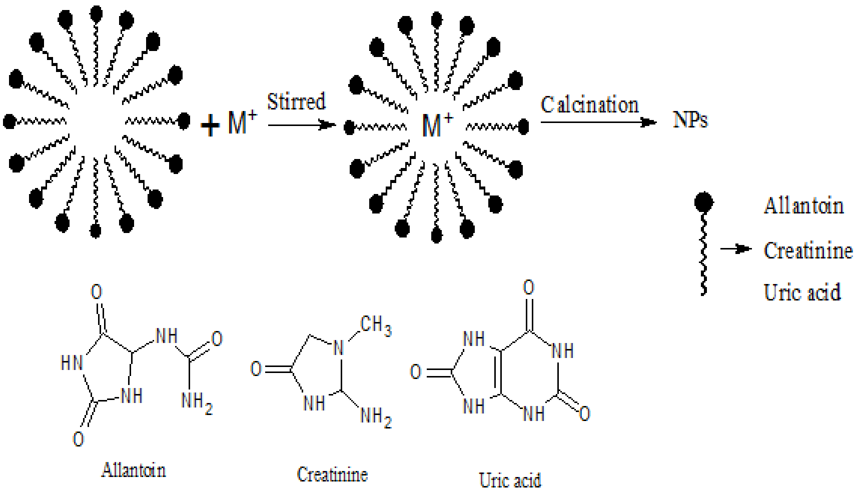


Fig. 5. Plausible mechanism of green synthesis of NPs using cow urine

with biomolecules which are existed in cow urine and the negative side of biomolecule gets pulled in the positive charge metal and this complex form stabilized through interaction. A few cow urine constituents were detailed in which biomolecules act as bio-reducing, stabilizing, and/or capping agents and the part of biomolecules depends on their structure and reactive sides as appeared in **Fig. 5**. The interaction results in the precipitation to form stable complexes and after calcination yields the desired NMs.

## CHARACTERIZATION TECHNIQUES FOR NMs

The biogenically synthesized NMs are commonly explored based on their stability, size, morphology, dispersity, and surface area using diverse characterization techniques [56-67]. UV-visible spectroscopic data, Fourier transform infrared spectroscopy (FT-IR), X-ray powder diffraction (XRD), X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), energy-dispersive X-ray spectroscopy

Table 2. Characterization techniques for NMs

| S. no. | Characterization techniques | Information obtained from the techniques |
|---|---|---|
| 1 | UV-Visible spectroscopy | Stability and formation of NPs |
| 2 | Thermo gravimetric analysis (TGA) | Thermal stability |
| 3 | X-ray powder diffraction (XRD) | Crystal structure and crystal size |
| 4 | Scanning electron microscopy (SEM) | Shape and size |
| 5 | Transmission electron microscopy (TEM) | Shape and size |
| 6 | Energy dispersive X-ray spectroscopy (EDS) | Elemental analysis and purity |
| 7 | Fourier transform infrared spectroscopy (FT-IR) | Functional group |
| 8 | Dynamic light scattering (DLS) | Size distribution of NPs |
| 9 | Atomic force microscopy (AFM) | Surface topography and NPs size |
| 10 | X-ray photoelectron spectroscopy (XPS) | Chemical surface analysis |
| 11 | Atomic absorption spectroscopy (AAS) | Elemental analysis |
| 12 | Vibrating sample magnetometer (VSM) | Magnetic properties of NPs |
| 13 | Brunauer-Emmett-Teller (BET) | Surface Area and porosity |
| 14 | Zeta Potential | Surface charge and stability |

(EDS), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images, dynamic light scattering (DLS), atomic absorption spectroscopy (AAS), vibrating sample magnetometer (VSM), Brunauer-Emmett-Teller (BET), thermogravimetric analysis (TGA), zeta potential and other characterization tools reveal the formation of green synthesized NMs and their growth, surface area, size, shape and magnetic properties [56-70]. **Table 2** summarizes some of the important techniques for the characterization of NMs.

## APPLICATIONS OF COW URINE MEDIATED NMs

Recently, cow urine mediated NMs may have versatile applications depending on the diverse features they manifest; therefore, we have portrayed their effective applications in anti-asthma, antimicrobial, antioxidant, anticancer, and photocatalytic activity to emphasize their momentous outcomes as guidance to upcoming researchers for future standpoints.

Babujanarthanam *et al.* [42] synthesized spherical-shaped aceprophyline cow urine nanoparticles (NPs) and reported their anti-asthma properties. The formation and stability of the NPs were affirmed by FT-IR, DSC, SEM, TEM, and UV methods.

The investigation over the chemical composition of diverse sorts of dairy animals' pee test is continuously a curiously subject, as examination appears that pregnant cow urine is more valuable although it contains ovarian hormones [55].

Meghnath Prabhu *et al.* [43] described the one-pot green synthesis of Ag NPs utilizing aged cow urine as a reducing agent and examined their antimicrobial activities. The rapid synthesis of Ag NPs was accomplished by sonication method at pH 9.5 for 1 min and stability, size, and morphology were explored by UV, FT-IR, XRD, SEM, ZP, and TEM. Moreover, as-synthesized Ag NPs exhibited good antimicrobial against *Staphylococcus aureus, Streptococcus epidermis, Bacillus subtilis, Escherichia coli, Salmonella typhi, Klebsiella pneumonia,* and *Fusarium oxysporum* using the disc diffusion method.

Jain *et al.* [44] reported the hydrothermal synthesis of Ag NPs using cow urine and honey and studied their antibacterial activities (**Fig. 6**). The synthesized Ag NPs evinced considerable antibacterial activity against bacterial strain *Pseudomonas sp*.

Muthusamy Govarthanan *et al.* [45] described the biosynthesis of spherical-shaped Ag NPs utilizing Panchagavya and silver nitrate. The formation of Ag NPs was affirmed by SEM (**Fig. 7**), TEM, and XRD techniques. The as-synthesized NPs showed considerable antibacterial activity against *Aeromonas sp., Acinetobacter sp.* and *Citrobacter sp.*

Similarly, Ranganathan et al. [46] reported the economic, simple, eco-friendly, and non-toxic Panchagavya mediated Cu NPs synthesis. Similarly, they studied the cytotoxicity performance of Cu NPs in brine shrimp.

Chandrasekhar et al. [47] described a facile approach for $Ag_2O$ NPs biosynthesis using cow urine without using any noxious substances and investigated their antibacterial and catalytic activities. These synthesized $Ag_2O$ NPs exhibited

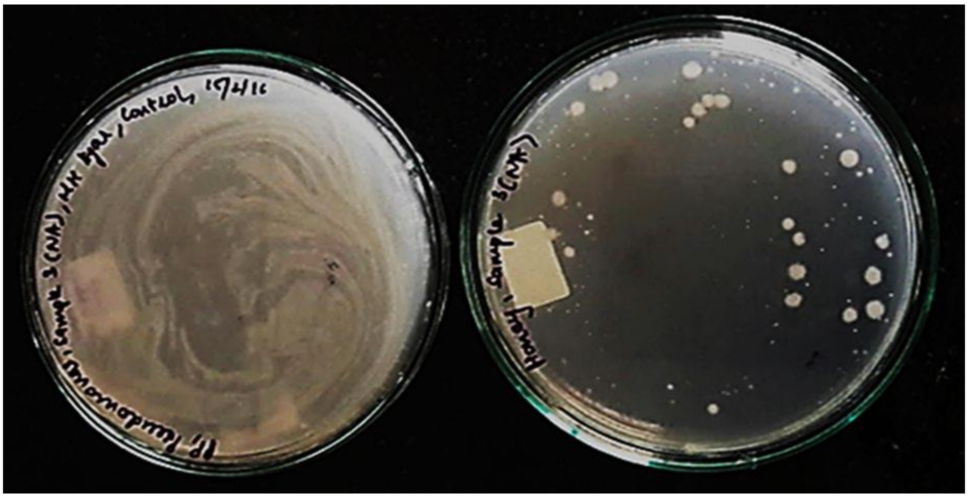


Fig. 6. Antibacterial activity of cow urine and honey mediated Ag NPs (Reproduced from ref. 44)

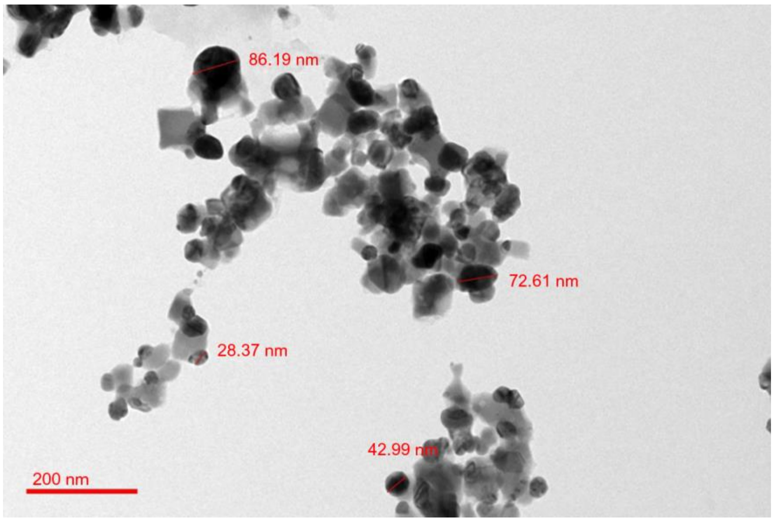


Fig. 7. Panchagavya mediated spherical shaped Ag NPs (Reproduced from ref. 45)

significant antibacterial activity against *Staphylococcus aureus, Pseudomonas desmolyticum, Escherichia coli,* and *Klebsiella aerogenes*. Moreover, they demonstrated that the photocatalytic degradation (**Fig. 8**) of methylene blue up to 83.63 % for 4 h by using $Ag_2O$ NPs as a photocatalyst.

The concentration-dependent study was described by Nazeruddin *et al.* [48], where Cd NPs were synthesized using cow urine and its effect towards a pathogenic bacterium, namely, *Staphylococcus aureus*, *Klebsiella pneumonia,* and *Pseudomonas aeruginosa* was studied.

Gopinath *et al.* [49] reported the cow urine encapsulated cellulose NPs using microemulsion method and examined antimicrobial activity of NPs against *Bacillus subtilis* and *Aspergillus niger* using disc diffusion assay.

Ranjith Kumar *et al.* [50] described the successful synthesis of spherical shaped $CuFe_2O_4$ NPs using the sol-gel method and cow urine was used as a chelating agent. The average size of the $CuFe_2O_4$ NPs is 14.5-22.3 nm.

Kar et al. [51] synthesized graphene nanosheets (GNs) using cow urine and urea. This synthesized GNs showed better electrical and optical performance.

Prasad et al. [54] described the bio-inspired synthesis of Pd NPs using cow excreta; similarly, they examined the antimicrobial, antioxidant, and catalytic activities of as-prepared NPs. These

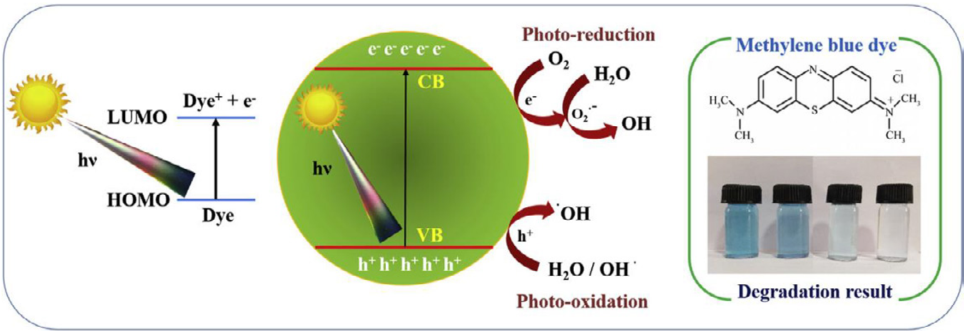


Fig. 8. Mechanism for the dye degradation using cow urine mediated $Ag_2O$ NPs (Reproduced from ref. 47)

synthesized Pd NPs possessed considerable antioxidant properties using ABTS and DPPH radical scavenging assay and indicated considerable antimicrobial activity against *Salmonella typhi, Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli, Fusarium solani,* and *Aspergillus niger* using the microdilution method. Besides, the synthesized Pd NPs showed excelled catalytic performance for the reduction of 4-nitrophenol into 4-aminophenol.

**FUTURE PROSPECTS**

Future research and modern advancements in the manufacturing of nanomaterials and novel, facile, non-noxious, economically affordable, and green nano-synthesis strategies should be directed toward improving laboratory-based research to an industrial scale with an enhanced awareness of health and environmental aspects in particular. Among diverse synthetic procedures for the production of nanomaterials, their synthesis using a natural, non-noxious source such as cow urine is developing as a crucial area and an eco-accommodating weapon in nanotechnology.

Cow urine, as a reducing and stabilizing agent, has many benefits of the green synthesis of nanomaterials. The prime reasons for this involve:

- Cow urine could be used as a reliable alternative for the quick, rapid, and one-pot synthesis of nanomaterial on a large scale.
- The production of nanomaterial using cow urine is an environmentally gracious method. The production rate of nanomaterial is faster than Physico-chemical protocols.
- For biological and medical applications where the purity of nanomaterials is of major concern, nanomaterials manufactured by cow urine are more eco-benign, stable, affordable, reliable, efficient, and free of chemical pollutants.
- Easy availability of cow urine.
- The use of high temperature, pressure, and energy for the green synthesis of nanomaterials is not required. These methods could also lead to energy savings and minimize the cost of nanomaterial production.

However, several problems still need to be tackled soon, considering the recent developments in the green synthesis of nanomaterials using cow urine:

- The precise mechanism by which the cow urine facilitates the production of nanomaterials is not completely studied yet.
- The effects on shape size and topography of the fabricated nanomaterials by varying reaction time and temperature have not been thoroughly considered; therefore, experimental trials would be needed to better understand it.
- When assessing the fabrication of nanomaterials using cow urine, monodispersity is a significant property; therefore, by studying the synthesis parameters such as the synthesis conditions, amount of cow urine, pH, temperature, reaction time, and effective monodispersity control is required.
- The biogenic synthesis of cow urine-mediated nanomaterials created mostly spherical-shaped particles; therefore, to control the morphology of the nanomaterials, factors such as the amount of cow urine, pH, temperature, reaction time, should be taken into consideration.
- The eco-friendly produced nanomaterials should be implemented to umpteen applications such as removal of pollutants, hydrogen production, biosensors, defense, ceramics, organic transformations, and multifunctional biological activities.

## CONCLUSION

This review article was covered to encompass the current research scenario of the green syntheses of NMs using cow urine as fuel and investigated their versatile applications. Expected synthesis mechanisms and an updated literature survey on the role of cow urine in syntheses have been explored. In summary, future research work viewpoint green NMs syntheses should be guided toward extending laboratory-based work to an industrial scale by considering current threats, particularly environmental effects and human health. However, green NMs syntheses based on biomolecules-assisted NMs is likely to be applied broadly both to the area of ecosystem remediation and in other important branches like biosensor, pharmaceutical, textile, food, ceramics, and cosmetic industries. Green syntheses of NMs using cow urine is an area that remains largely unexplored. Accordingly, plentiful possibilities remain for the exploration of new green preparatory protocols dependent on biosynthesis.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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