Engineering and Applied Science Letters

PSR Press

Article

Emerging trends of nanomaterials synthesis using bos taurus urine

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Received: 07 April 2024; Accepted: 12 June 2024; Published: 30 June 2024.

Abstract: The ability of organisms or organic compounds to reduce metal ions and stabilize them into nanoparticles is known as green synthesis. Various synthesis methods have been developed, each with its own advantages and drawbacks. In recent years, nanomaterials have found extensive applications in biological sciences, particularly in health and veterinary medicine. For these applications, it is crucial that nanomaterials are biocompatible and non-toxic. Consequently, researchers have increasingly focused on biological synthesis routes. Drawing inspiration from the ancient Indian system of medicine, Ayurveda, some researchers have recently synthesized nanomaterials using Indian cow urine. This review aims to catalog the various nanomaterials produced using Indian cow urine and to discuss their catalytic and biological activities.

Keywords: Bos taurus urine; Bio-synthesis; Nanomaterials; Biological activity; Catalytic efficacy

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1. Introduction

Since the dawn of civilization, the researchers working in the domain of material science are trying to develop novel materials with exotic properties. The developmental scenario of human civilization is many times recognized after the name of peculiar materials used in that particular period, for example stone age, iron age, copper age, plastic age and now stepped into Nano age. The present and future challenges and opportunities in the field of material science and technology are more exciting than those of past [1]. The term nanometer was first used in 1914 by Richard Zsigmondy. The idea of potential of science at nanoscale was recognized by Nobel laureate Richard Feynman. In his speech on 29 December 1959, entitled 'There is plenty of room at the bottom' he imagined that entire Encyclopedia Britannica could be written on the head of a pin [2]. This is considered to be first academic talk on nanotechnology. Actually, at that time the idea was digested in the mind of researchers. The term Nanotechnology was coined by Japanese scientist Nario Taniguchi in 1974 [3]. But today we observe it is a reality, as we can easily store the books in small pen-drive. Here the properties of materials are size-dependent. Thus, when particle size is made to be nanoscale, various properties such as electrical conductivity, magnetic permeability, chemical reactivity, melting point etc. changes as a function of the size of particles [4,5]. This led to the development of new branch of science known as nanoscience and technology. Today the researchers working in the area of material science are trying to develop materials at Nanoscale. Many new devices and patentable technology is based on nanomaterials. The astonishing properties of nanomaterials makes it enable to effectively apply in different areas such as biosensors, Nano-catalyst, chemical sensors, super-capacitors, veterinary medicine and food science etc. First of all, nanoscale materials are not new to science. They already exist, but the exact history of the utilization of Nano-sized objects by human is difficult to clarify. However, the basic history of nanoscale material utilization is from ancient times and human being used such materials for various applications though the term Nano was not used at that time. The properties of materials at Nano scale differs from that of bulk. Their properties were not studied due to lack of instrumentation. The properties of materials at nanoscale are being explored due to microscopic engineering and technological revolution day by day [6]. The phenomenon at nanoscale based on quantum effects and other simple physical effects such as increase in surface area to volume with respect to their bulk counterparts. This is because at macroscopic level, materials are surrounded by similar atoms/ molecules in all directions. But at Nano-scale, this surrounding is only lateral. Therefore, physical properties change drastically. At macroscale, only strong forces and external influences play a role in the observed properties. At nanoscale forces can be considered as short-range forces with interaction distance less than a few Nano-meter. At nanoscale, weak forces and quantum mechanical effects as well as uncertainty come into play, which cause anomalies in the observed properties [7]. Secondly, when we go on reducing the size of materials surface to volume ratio becomes high. Most measurable properties of particles are influenced by more than one of the fundamental properties. Thus, the measured properties are function of surface/ volume ratio. Another reason is free electron path. In nanomaterials the free electron path is bigger than the actual particle resulting in enhanced plasmonic properties with all the consequences for electrical and optical properties [8]. Properties of conducting material strongly depends upon band gap. As a matter of fact, the band gap in metals are overlapping so they are best conductors, then there is small band gap in case of semi-conductors. In case of insulators there is wide band gap. Now, what happens when the materials are taken to Nano meter level. When the material is reduced to Nano meter level, the particles gets discrete in nature and they become dominated by phenomenon known as quantum effects and other simple physical effects such as expanded surface area [9]. Due to expanded surface more and more atoms are required to fill the band and hence band gap widens. So the material in bulk which was a metal may behave like an insulator or semi-conductor. The main properties of the bulk materials which differs at nanoscale are optical, electrical, physical and chemical. Therefore, due to astonishing properties of materials at nanoscale there is growing interest in the mind of researchers to design and develop materials at nanoscale Nanotechnology is the new field of science that is evolved in 20th century. Due to small size, these nanomaterials have novel properties and functions in every field of science [10]. According to Nario Taniguchi, 'Nanotechnology consists of the processing, separation, consolidation and deformation of materials by one atom or one molecule. Though the concept was nanoscience and technology were seeded by Richard Feynman, before 1980s, nanotechnology remained an area for discussion only. The concept could come into actual practice after the discovery and availability of electron microscope. The properties of materials nanoscale depend upon its size and shape. The electron microscope micrograph reflects the images of the materials with high magnifications and resolutions. The properties of materials at nanoscale differs from that of bulk, this is because nanoscale materials have high aspect ratio, gravitational force becomes ineffective, electromagnetic force of attraction or repulsion becomes significant [11]. When we look at phenomenon at bulk and nanoscale, we find that properties at bulk is the average properties, whereas properties at nanoscale is the property at that particular scale. Hence there is wide deviation in the properties at nanoscale and could be successfully implemented in different domains [12]. Recently nanomaterials are being used in veterinary medicine, food and animal nutrition, anti-microbial agents and so on.

2. Synthesis of Nanoparticles

The nanostructured materials may have been suitable candidates in one application but could be more useful in a different application if synthesized by different mechanism and technique. Also, the structure and morphology of materials at nanoscale depend upon method of growth and synthesis. Thus, synthesis plays a crucial role in properties of materials at nanoscale. The synthetic route can be broadly classified into two categories such as (1) Top-down route and (2) Bottom up route of synthesis [13,14]. Top-Down route of Synthesis: This method is easy to understand and digest. In top-down approach, bulk materials are divided into smaller parts so as to produce materials at nanoscale. The top-down method involves mechanical ball milling, sputtering, etching, laser ablation, physical vapor deposition, electro-explosion etc.

2.1. Different approaches (Top down and bottom-up approach for NP synthesis)

As demonstrated below in Figure 1, synthesizing route of nanomaterials can be classified into two main types such as (1) Top-down route and (2) Bottom up method. In the earlier attempts the nanomaterials could be synthesized from top-down routes of synthesis. The mechanical ball milling is the most facile and easy to understand route of synthesis. Here due to mechanical impacts of balls on the micro sized materials, they are converted into Nano range. Though this route of synthesis is easy and materials can be produced at large scale this method suffers from many drawbacks such as the uneven size of nanomaterials. Also there are imperfections and defects in the synthesized materials. Therefore, the researchers turned towards

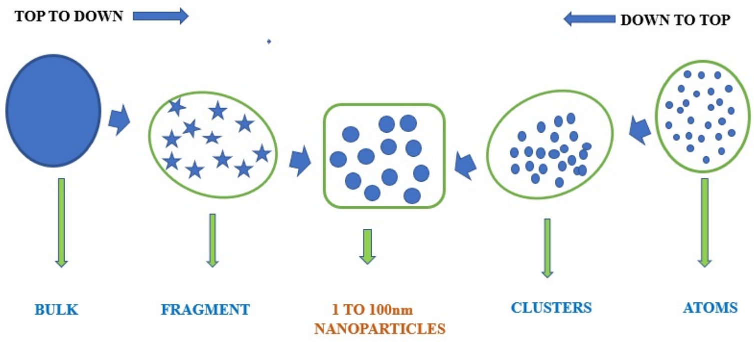

Figure 1. Schematic representation of top down and bottom-up approach

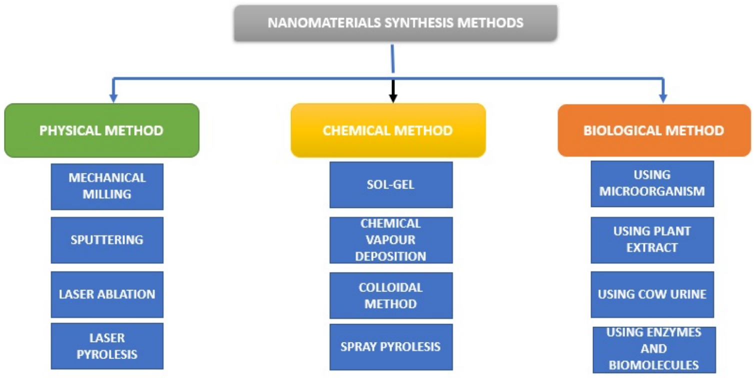

Figure 2. Various routes for the synthesis of nanoparticles

another route of synthesis [15]. Physical and chemical treatments are used to reduce the size of the object. Metal vapor deposition techniques are an example of laboratory synthesis utilizing a top-down approach. However, this technology has few disadvantages, including the difficulty of obtaining a tight particle size distribution and the high difficulty of installing metal vapor machines. Another disadvantage of physical techniques is the considerable energy consumption required to sustain the high temperature and pressure required for the synthesis step. Most bioprocesses, on the other hand, take place at normal air pressure and temperature, resulting in significant energy savings. Because the surface chemistry and other physical attributes of nanoparticles are heavily reliant on the surface structure, top-down manufacturing methods produce defects in the product's surface structure, which is a severe restriction [16].

3. Varius Routes of Synthesis of Nano Particles

In top-down method of synthesis of nanoparticles bulk materials are converted to nanoparticles using various methods such as, Mechanical milling, nanolithography, laser ablation, sputtering and thermal decomposition are some of the most widely used nanoparticle synthesis methods. On the other hand, in bottom-up method nanoparticles are built by clusters of atoms. Sol-gel, spinning, chemical vapor deposition (CVD), pyrolysis and biosynthesis are the most commonly used bottom-up methods for nanoparticle production [17,18]. In the earlier stage the researchers used microbes to synthesize nanomaterials. But soon it was realized that maintaining the microbes requires good aseptic conditions and hence its cost increases. Then, the researchers turned to use various plant extracts. Using various plant extracts silver nanomaterials could be very successfully synthesized. Then our research group tried to develop another route of synthesis i.e. using Indian cow urine

3.0.1. Physical And Chemical Method Of Synthesis Of Nanoparticles

Herein we would like to mention the developmental scenario of synthesis of nanomaterials. In the beginning attempts were made to synthesize nanomaterials through physical route of synthesis. The physical route of synthesis is very simple to understand. But the physical route of synthesis have certain limitations

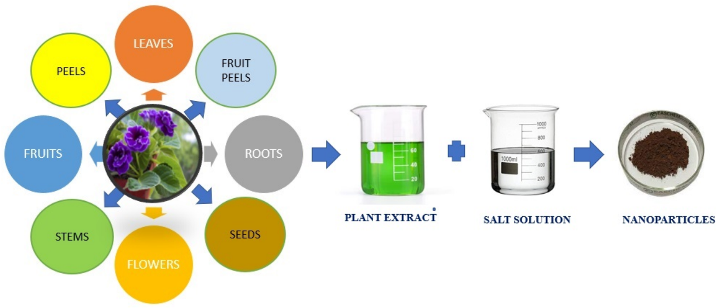

Figure 3. Synthesis of nanoparticles using plant extract

such as it requires need to expensive instructions which is not available commonly. Further, the nanomaterials synthesis through physical route of synthesis suffers from defects and generally it is very difficult to get monodispersed nanomaterials. In order to overcome these limitations, another route of synthesis i.e. chemical route of synthesis was developed. Although chemical route of synthesis could bypass the drawbacks of earlier methods. However, the chemical route of synthesis uses hazardous and expensive chemicals. Therefore, many times the nanomaterials of biological interest is not advisable. To overcome these limitations, researchers tried to switch on towards new method of synthesis i.e. biological route of synthesis

3.1. Biosynthesis Of Nanoparticles

Biosynthesis is a green, sustainable and environmentally friendly cost-effective approach for the synthesis of nanoparticles [19]. Apart from this, the biosynthesis of nanoparticles has the better control over the crystal growth i.e. we can adjust the size and shape of nanomaterials by monitoring the reaction conditions and choice of biological reducing agents and capping agents. It is interesting to mention that most of biosynthesis can take place at physiological conditions of temperature, Ph etc. In the earlier attempts through biosynthesis microbes were used. The microbes could effectively reduce metal ions and produce nanomaterials in zero oxidation state. But soon it was realized that the microbial route of synthesis requires maintenance of culture medium and highly aseptic conditions. Further, the microbial synthesis requires highly expertise manpower which is not always available. In order to overcome these limitations researchers turned towards other route of synthesis i.e. using various plant extracts

3.1.1. Synthesis Of Nanoparticles Using Plant Extract

As we have discussed hereon, the researchers working in the domain of nanoscience and technology tried to synthesize materials at nanoscale using various plant extracts. It is known to us that plants contain various anti-oxidants. These anti-oxidants are nothing but natural reducing agents. The commonly found anti-oxidants in plants are flavonoids, polyphenol, terpenoids, isoprene etc. Also many medicinally and aromatically important plants contain alkaloids. The alkaloids are heterocyclic Nitrogen containing organic compounds. Here electron can be donated during the course of bio-chemical reactions. Also various protein moieties present in the plant extract may act as suitable capping agent which imparts stability to the nanomaterials. Within the few years after invention, the biological route of synthesis using plant extract became very popular among the researchers and academicians. During last few decades several research papers are published which indicates biological synthesis of nanomaterials using plant extracts. This route of synthesis is popular even today and researchers are trying to see its effectiveness for different metals and also bimetallic material synthesis. The plant extract mediated synthesis of nanomaterials uses various parts of the plants such as leaves, roots, flowers, pollens, stem etc. The biomimetic synthesis of nanoparticles using plant extracts can be depicted diagrammatically as below in Figure 3. In fact, biological route of synthesis using plant extract can produce biocompatible nanomaterials in economic ways. But we would like to discuss some limitations of biological route of synthesis using plant extract. Here we are using various plant extracts. This may harm the flora and fauna. Also, some plants are rare and have high medicinal importance. Hence, it is better if these limitations could be overcome.

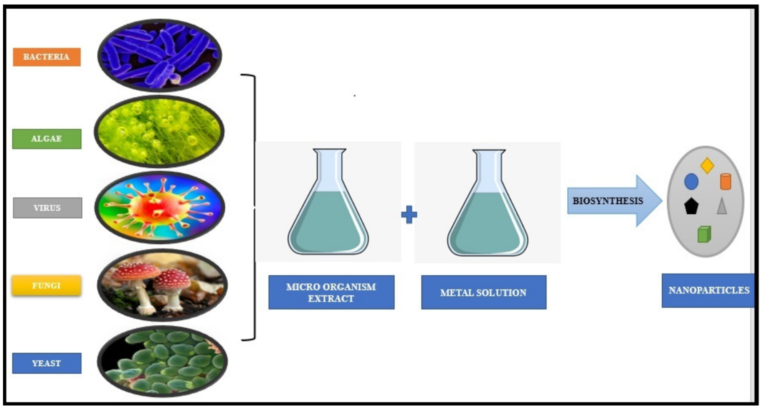

Figure 4. Biosynthesis of nanoparticles by using microorganism

3.1.2. Biosynthesis Of Nanoparticles Using Microorganism

Biosynthesis of nanomaterials using microorganism can be divided into two types according to the location where nanoparticles are formed namely intracellular and extracellular synthesis. [25]. The intracellular method consists of transporting ions into the microbial cell to form nanoparticles in the presence of enzymes. The extracellular synthesis of nanoparticles involves trapping the metal ions on the surface of the cells and reducing ions in the presence of enzymes. [26] Various types of nanomaterials synthesized using different microorganism such as bacteria, fungi, algae, yeast, virus etc. as shown in Figure 4.

3.1.3. Synthesis Of Nanoparticles Using Cow Urine

After thorough analysis of all the research papers published by various researchers and brain storm thinking we feel that the scholars who developed biosynthesis of nanoparticles using Indian cow urine are highly influenced by Ayurveda. Ayurveda is the ancient system of holistic medicine developed in Indian subcontinent. It is supposed to be originated from ancient Indian epic Atharvaveda. Ayurveda is the ancient science of holistic medicine developed in Indian sub-continent. Ayurveda is supposed to be originated from ancient Indian book Atharvaveda. The Ayurveda system of medicine. In Ayurveda system of medicine various plant based products, inorganic minerals – also metals are used for treatment. Apart from this Ayurveda literature describes the medicinal importance of Indian cow urine, cow milk, clarified butter, butter milk etc. Herein taking inspiration from ancient Indian literature our research group and similarly other researchers tried to synthesize nanomaterials using Indian cow urine [27]. Extensive research work has been done now and early in Indian subcontinent on the medicinal properties of cow products. The Ayurvedic medicines can be classified into three categories (1) plant based, (2) animal based and (3) inorganic compounds, metals, minerals after various stepwise treatment. Taking inspiration from to ancient Indian literature our research group and several other researchers tried implement it in the domain of nanomaterials formation. The Atharvaveda recognized cow as earthy form of Lord Vishnu. The cow is gifted with the natural power of universe and it passes on these energies through its by products such as milk, cow dung and cow urine. According to Ayurvedic literature cow urine is capable to cure peptic ulcer, liver ailments, psoriasis, anemia, fever, leprosy and even cancer. The author is having personal experience of effectiveness of cow urine against constipation. This is the sure shot medicine for indigestion. According to ancient Indian literature Sushruta Samhita and Ashtanga Sangraha, Rajnighunt, Amritsagar cow urine is the most effective substance/ secretion of animal origin with innumerable therapeutic values and considered as divine medicine which can be used for treatment of diabetes, blood pressure, prostate enlargement, ulcer, gynecological problems [28] Thus consumption of cow urine helps to cure incurable diseases and auto-immune disorders. Therefore, cow urine has been granted patents for its antibiotic, antifungal, anti-cancer and bio-enhancer properties [29]. Since cow urine is fortified with various salts, enzymes and micro-nutrients, drinking cow urine or adding it to health drink and concoctions imparts numerous health benefits. Ayurveda literature describes the use of cow products for medicinal purpose. Liquid metabolic waste of cow is a constituent of Panchagavya (a combination of cow urine, milk, clarified butter, curd and dung). By considering all these aspects, we realize that biosynthesis of nanoparticles using Indian cow urine is most effective, eco-friendly route of synthesis. But

Table 1. Chemical composition and health effects of cow urine

S. No.	Chemicals fund in cow urine	Positive Effects on Health
1	Ammonia	Improves formation of blood. The three properties of body bile, mucus and air is stabilized
2	Aurum hydroxide	It imparts immunity, it acts as germicide, antibiotic etc
3	Calcium	It strength the bone and important constituent of skeletal systems
4.	Phenol	It acts as antiseptic and can control gangrene
5.	Creatinine	It acts as effective germicide
6	Enzymes	Improves digestion and immunity
7	Hipuric acid	It accelerates removal of toxins
8.	Iron	It assists in formation of RBCs and hemoglobin
9.	Lactose	It strengthens heart and controls nervousness
10.	Nitrogen	It acts as diuretic
11.	Sodium	It helps in purification of blood

we realize in spite of these advantage this route of synthesis is yet not become very popular among scientific community. Hence an effort has been made to popularize it among the researchers working in Nanoscience and Technology. Chemical composition of Cow Urine: The physico-chemical properties of urine is studied for various purposes e.g. diagnosis of the illness. Cow urine is the physiologically processed liquid metabolic waste discharged naturally and regularly as a result of proper function of excretory systems. The animals can discharge nitrogenous waste in various forms such as ammonia, urea and uric acid. The aquatic animals and amphibians excrete nitrogenous waste in the form of ammonia which is highly soluble in waster, the terrestrial animals like human, cow etc. excretes nitrogenous waste in the form of urea, whereas avian excrete nitrogenous waste in the form of uric acid. The main chemical composition of urine is of course water which is present about 95% and is followed by urea which is about 2.5%. And the remaining 2.5% part contains various minerals, salts, hormones, enzymes, proteins, bile salt, bile pigment etc. Urea is the second largest compound present in urine, therefore the name urea is derived. The cow urine shows the effective antimicrobial activity and germicidal activity. This may be due to presence of urea, aurum hydroxide, creatinine, carbolic acid and calcium and manganese salts. Cow urine shows another important property i.e. anti-neoplastic activity. This is the property which suppresses tumors to grow. Today in Ayurveda cow urine is effectively used to treat some forms of cancer. The antineoplastic activity may be due to presence of uric acid and aurum hydroxide in cow urine. Aurum hydroxide improves immunity and allantoin striges healing of the wound. Therefore, Ayurveda considers cow urine as the live dispensary and power house of medicines. Herein we take an effort to focus on the health benefits of cow urine.

So far, we have seen the positive health effects of cow urine. The health-related benefits of cow urine are known since ancient time. But with the advancement in the scientific and modern technological knowledge, researchers are trying to search novel methods for the synthesis of materials at nanoscale. When we peep into literature, we observe several scholars have successfully synthesized transition metal and metal oxide nanoparticles using Indian cow urine. By considering the economical, biological and environmental significance of biosynthesis of nanomaterials using Indian cow urine, we realize that it is most efficient route of synthesis of nanomaterials. Even though we observe that this route of synthesis is yet not very popular among the scientific community. Hence an effort has been made to popularize it among the researchers working in Nanoscience and Technology. Herein an attempt has been made to enlist various nanomaterials synthesized using Indian cow urine. The nanoparticles prepared from cow urine are mentioned in Table 2

Table 2. Synthesis of Nanoparticles Using Cow Urine

Sr. No.	Nanoparticles	Shape	Size (Nanometer)	Precursors	References
1	Silver (Ag)	Spherical	29.92	AgNO3	[30]
2	Silver (Ag)	Face Centered Cubic	11	AgNO3	[31]
3	Copper (Cu)	Spherical	98	CuSO4	[32]
4	Graphene nanosheet	Nanosheet	-	Graphene oxide	[33]
5	Copper Oxide	Spherical	-	CuSO4	[34]
6	Palladium	Cylindrical	-	Palladium chloride	[35]
7	Silver (Ag)	Spherical	20-100	AgNO3	[36]
8	Silver (Ag)	-	-	AgNO3	[37]
9	Silver Oxide	Spherical	20	AgNO3	[38]
10	Copper Ferrite (CuFe2O4)	Spherical	14.5-22.3	Cu(NO3)26H2O Fe(NO3)9H2	[39]
11	Graphene nanosheet	Nanosheet	-	Natural Flake Graphite (NFG)	[40]
12	Cellulose	Spherical	30-40	Cellulose	[41]
13	Graphene	Nanosheet	3	Graphene Oxide	[42]
14	Zinc Oxide (ZnO)		3.99		[43]
15	Cobalt Oxide (CoO)	Spherical	100-400	CoCl2.6H2O	[44]
16	Gold (Au)	Cubic	12	HAuCl4	[45]
17	Cadmium	Cubic	62.2	CdSO4	[46]

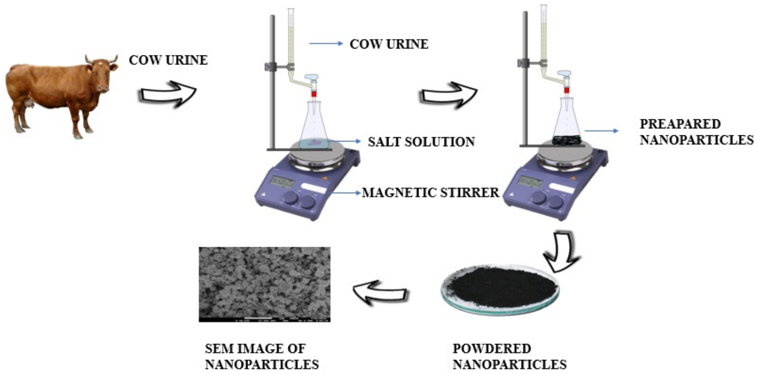

Figure 5. Synthesis of nanoparticles using cow urine

4. Methodology for Synthesis of Nanomaterials

In the green synthesis of NMs, commonly metal particle comes in contact 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 [47] . The transition metal and metal oxide nanoparticles can be effectively synthesized using Indian cow urine. For the biosynthesis of nanoparticles using Indian cow urine, first the freshly discharged liquid metabolic waste of vaccinated and healthy Indian cow is collected in well sterilized glass pot. The liquid metabolic waste of Indian cow urine was dribbled using filter paper and stored in suitable container at room temperature. Then corresponding salt solution prepared in double distilled water. The stabilizing agent or capping agent dissolved in double distilled water and slowly added in above solution. These reaction mixtures titrate against cow urine with constant stirring. The precipitate appeared in the reaction mixture which is continuously heated till complete dry. Then the solid mass remaining in the beaker was separated with the help of spatula and crushed in fine power in mortar which is further used for characterization various catalytic and biological applications. This process can be represented diagrammatically in Figure 5.

5. Characterization

At the end of reaction process, we get fine power of materials. Now, it becomes essential to determine which material is formed, what is its morphology i.e. how it looks and its properties. As nanomaterials are beyond the perception of human eyes, it becomes impossible to visualize its morphology and nature. Hence it becomes essential to characterize the synthesized materials using advanced characterization techniques. Till date no chemical route of analysis is in practice. Hence, we have to rely on the physical methods of analysis. The researchers freely use physical techniques for analysis of nanomaterials. The physical analysis method is expensive and also needs expert hands to handle. Modern instruments can accurately determine the size of materials and electron micrograms can reveal the structure of nanomaterials. In many advanced research laboratories these facilities are now available. The characterization technique includes UV- visible analysis, XRD, Electron microscopic techniques, Zeta potential and DLS etc. [48]. These characterization techniques give specific information about nanoparticles which is summarized in Table 2. [49]

Table 3. Glimpses on Experimental Techniques That Are Used for Nanomaterial Characterization

Abbreviation	**Characterization Techniques**	Main Information (Utility)
XRD	X-Ray Diffraction	Crystal structure, composition, crystallite size,
XAS	X-Ray Absorption Spectroscopy	X-ray absorption co-efficient, chemical state of species, interatomic distances, Debye-Waller factors, and non-crystalline NPs
SAXS	Small Angle X-Ray Scattering	Particle size, size distribution, growth kinetics
XPS	X-ray photoelectron Spectroscopy	Electronic structure, elemental composition, oxidation states, ligand binding
FT-IR	Fourier Transform Infrared Spectroscopy	Surface composition, ligand binding
NMR	Nuclear Magnetic Resonance Spectroscopy	Ligand density and arrangements, electronic core structure, atomic composition, the influence of ligands on NP shape, NP size
BET	Brunauer Emmett Teller	Surface area
TGA	Thermogravimetric Analysis	Mass and composition of stabilizers
LEIS	Low Energy Ion Scattering	Thickness and chemical composition of self-assembled monolayers of NPs
UV-Visible spectroscopy	Ultra-violet Visible Spectroscopy	Optical properties, size, concentration, and agglomeration state, hints at nanoparticles shape
PL Spectroscopy	Photoluminescence spectroscopy	Optical properties, relation to structural features such as defects, size, composition, etc
DLS	Dynamic Light Scattering	Hydrodynamic size, detection of agglomeration
NTA	Nanoparticle Tracking Analysis	Nanoparticles size and their distribution
DCA	Direct Coupling Analysis	Nanoparticles size and their distribution
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy	Elemental composition, size, size distribution, NP concentration
SIMS, ToF-SIMS, MALDI	Sputtering Ion Mass Spectroscopy	Chemical information on functional groups especially surface sensitivity, molecular orientation, and conformation, surface topography, MALDI for nanoparticle size
VSM	Vibrating Sample Magnetometer	Magnetic properties of nanomaterials
Contact Angle	Contact Angle	Determination of hydrophobic characters of thin films

Continued on next page

Table 3 – continued from previous page

Time (s)	Triple chosen	Other feasible triples
FMR	Ferromagnetic Resonance Spectroscopy	Nanoparticle size and distribution, shape, crystallographic imperfections, surface composition, M value, magnetic anisotropic constant, demagnetization fields
XMCD	X-Ray Magnetic Circular Dichroism	Site symmetry and magnetic moments of transition metal ions in ferro and ferrimagnetic materials element-specific
CLSM	Confocal Laser Scanning Microscope	Imaging, ultrafine morphology
BAM	Brewster Angle Microscope	Gas-liquid interface imaging
APM	Atomic Probe Microscopy	Three-Dimensional Imaging
MFM	Magnetic Force Microscopy	Magnetic Material Analysis
Low Energy Electron Diffraction	Low Energy Electron Diffraction	Surface/Adsorbate bonding
AEM	Auger Electron Microscopy	Chemical Surface Analysis
CFM	Chemical Force Microscopy	Chemical/Surface Analysis
FIM	Field Ion Microscopy	Chemical Profile/ Atomic spacing
UPS	Ultraviolet Photoemission Spectroscopy	Surface Analysis
AAS	Atomic Absorption Spectroscopy	Chemical Analysis
ICM	Inductively Coupled Microscopy	Elemental Analysis
SANS	Small Angle Neutron Scattering	Surface Characterization
CL	Cthodoluminescence	Characteristic Emission
Nanocalorimetry	Nanocalorimetry	Latent Heat of Fusion
Sears Method	Sears Method	Colloidal size, specific surface area
FS	Fluorescent Spectroscopy	Elemental Analysis
LSPR	Localized Surface Plasmon Resonance	Nanosized particle Analysis
Rutherford Backscattering	Rutherford Backscattering	Quantitative Elemental Analysis
TEM	Transmission Electron Microscopy	NP size, size monodispersity shape, aggregation state, detect and localize quantify nanoparticles in matrices, study growth kinetics
HRTEM	High-Resolution Transmission Electron Microscopy	All information by conventional TEM and also on the crystal structure of a single particle. It is used to distinguish between monocrystalline, polycrystalline, and amorphous
Liquid TEM	Liquid Transmission Electron Microscopy	Depict nanoparticle growth in real-time, study growth mechanism, single particle motion, and superlattice formation
Cryo-TEM	Cryo Transmission Electron Microscopy	Study complex growth mechanisms, and aggregation pathways, good for molecular biology and colloidal chemistry to avoid the presence of artifacts or destroyed samples
ED	Electron Diffraction	Crystal structure, lattice parameter, study order, and disorder transformation, long-range order parameters
STEM	Scanning Transmission Electron Microscopy	Combined with HAADF, and EDX for morphology study, crystal structure, and elemental composition, Study the atomic structure of hetero-interface
Aberration- corrected (STEM, TEM)	Aberration corrected Scanning Transmission Electron Microscopy	Atomic structure of NP clusters, especially bimetallic ones, as a function of composition, alloy, homogeneity, phase segregation
EELS	Electron Energy Loss Spectroscopy	Type and quantity of atoms present, chemical states of atoms, collective interaction of atoms with neighbors, bulk plasma resonance
Electron tomography	Electron tomography	Realistic 3D particle visualization, snapshots, video, and quantitative information down to atomic scale

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Table 4. Nanoscale Parameters Characterization Techniques

Size (structural properties)	TEM, XRD, DLS, NTA, SAXS, HRTEM, SEM, AFM, EXAFM, FMR, DCS, ICP-MS, UV-Vis, MALDI, NMR, TRPS, EPLS, magnetic susceptibility
Shape	TEM, HRTEM, AFM, EPLS, FMR, 3D-tomography
Elemental chemical composition	XRD, XPS, ICP-MS, ICP-OES, SEM-EDX, NMR, MFM, LEIS
Crystal structure	XRD, EXAFS, HRTEM, STEM, electron diffraction
Size distribution	DCS, DLS, SAXS, NTA, ICP-MS, FMR, DTA, TRPS, SEM, superparamagnetic relaxometry
Magnetic properties	SQUID, VSM, MFM, FMR, XMCD, magnetic susceptibility
Optical properties	UV-Visible spectroscopy, Photoluminescence spectroscopy, EELS-STEM
Detection of Nanoparticles	TEM, SEM, STEM, EBSD, magnetic susceptibility
Structural defects	HRTEM, EBSD
Dispersion of nanoparticles in matrices	SEM, AFM, TEM
3D visualization	3D topography, AFM, SEM
Single-particle properties	Sp-ICP-MS, UV-Vis, RMM-MEMS, PTA, DCS, TRPS
Density	DCS, RMM-MEMS
Agglomeration state	Zeta potential, DLS, DCS, UV-Visible spectroscopy, SEM, Cryo-TEM, TEM
Concentration	ICP-MS, UV-Visible, RMM-MEMS, PTA, DCS, TRPS
Surface charges	Zeta potential, EPM
Surface area, specific surface area	BET, liquid NMR
Ligand binding/ composition/ density/ arrangement/ mass, surface composition	XPS, FTIR, NMR, SIMS, FMR, TGA, SANS
Growth kinetics	SAXS, NMR, TEM, Cro-TEM, liquid-TEM
Chemical state –oxidation state	XAS, EELS, XPS, Mossbauer

Table 3 – continued from previous page

Time (s)	Triple chosen	Other feasible triples
SEM-HRSEM, T-SE-EDX	Scanning Electron Microscopy- High-Resolution Scanning Electron Microscope	Morphology, dispersion of nanoparticles in cells and other matrices/ supports, precision in the lateral dimension of nanoparticles, quick examination-elemental composition
EBSD	Electron Backscattered Diffraction Microscopy	Structure, crystal orientation, and phase of matrices in SEM. Examine microstructure, reveal texture, defects, grain morphology, deformation
AFM	Atomic Force Microscope	Nanoparticle size and shape in 3D mode, evaluate the degree of covering of a surface with nanoparticle morphology, dispersion of nanoparticles in cell and other matrices/ supports, precision in the lateral dimension of nanoparticles, quick examination- elemental composition
MFM	Magnetic Force Microscopy	Standard AFM imaging together with the information of magnetic moments of single NPs. Study magnetic NPs in the interior of cells. Discriminate from non-magnetic NPs

6. Applications

In last few decades' major research in physics and chemistry took place in material synthesis, characterization and applications. In recent years, the transition metal and metal oxides especially noble metals like silver, gold and platinum have been extensively studied because of their unique properties like high aspect ratio. These nanomaterials find vast applications in various biomedical, engineering, industrial sectors and are also scientifically very important. The synthesized nanomaterials could be effectively used in drug delivery cancer therapy, bio-imaging, Nano-sensors, catalyst, dye degradation and many more. Herein we try to enlist

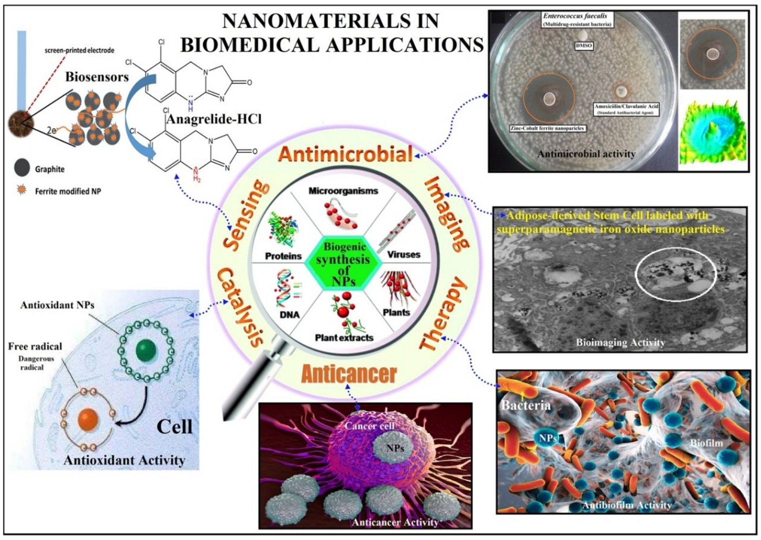

Figure 6. Biomedical Application of Nano Materials

the biomedical applications of nanomaterials. Bio-synthesis nanoparticles have various biomedical application as shown in Figure 6.

Nanoparticles have many potential applications in the field of biomedicine due to their unique properties such as their small size, large surface area, and ability to interact with biological molecules. Metal and metal oxides nanoparticles have shown great potentials for bio medicals applications such as Drugs delivery, Bio-imaging, Cancer therapy and antimicrobial activity.

[1] **Drug delivery:** Drug can be defined as agent other than food which can alter the physiological activity of an individual. Actually, when we are using drugs for therapeutic purposes it is called as medicinal drug. It is desired that the drug should act only on the particulate site, should not show any side effects, should be used in minimum quantity, should be excreted completely and should not damage healthy parts of the body. Today no drug is a ideal drug which have all these properties. But with the innovation of nanotechnology and particularly its effectiveness in drug delivery system, new doors of hopes are now opened. Nanomaterials can be used to deliver drugs to specific cell or tissues in the body. The encapsulating drugs within nanoparticles, it is possible to improve their solubility, stability and bio ability as well as to target them to specific cells.

[2] **Bio imaging:** Imaging is the non-invasive diagnostic technique. Today various types of imaging techniques like CT-Scan, MRI Scan etc. are widely used for imaging purposes. These radiological methods are extensively used in veterinary and human medicine. It is worth to mention that nanomaterials can be effectively used in various imaging techniques such as magnetic resonance imaging (MIR).

[3] **Cancer Therapy:** This is the uncontrolled proliferation of the cells or tissue. This is a fatal disease and most deadly cancer is glioblastoma multiferum. Today in allopathic ways of treatment cancer is treated by surgery, radiotherapy and chemotherapy. The chemotherapeutic drugs affect all the body parts and organs. Hence for cancer therapy targeted cancer therapy is always desired. Nanomaterials can be used for targeted cancer therapy by attaching targeting molecules to the surface of Nano particles. It is possible to selectively deliver drugs or other theopathic agent to cancer cells.

[4] **Bio-Sensor:** Sensors are instruments or materials which can convert physical changes into electrical signals. Today in majority of diagnostic tools various types of sensors are used. The commonly known example of biosensor is glucose sensors. Nanomaterials can be used to create bio sensor for the detection of bio molecules with high sensitivity and specifically.

[5] **Anti-microbial:** Nano materials are used as anti-microbial agent in various medical field. Since, nano materials have many applications in various fields, here we summarized some of the applications of nano particles synthesized by using cow urine given by some researchers.

P.D. Sarvalkar et al shows that silver nanoparticles synthesized by using cow urine act as nano catalyst for simple organic reactions such as conversion of 4-nitrophenol into 4-aminophenol, conversion of 2-nitroaniline to 2-Aminoaniline, conversion of 3-Nitroaniline to 3-Nitroaminoaniline and 4- Nitroaniline to 4-Nitroaniline. Also, they showed silver nanoparticles act as nano catalyst for the degradation reaction of Methylene Blue

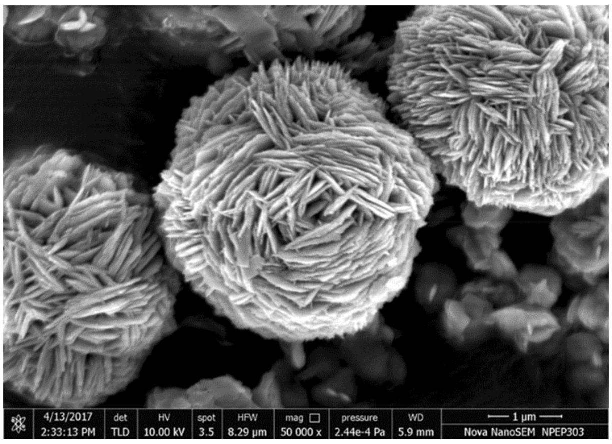

Figure 7. SEM image of Palladium nanoparticles. [ref :S. R. Prasad 2020]

and Crystal Voilet using UV light. M. Prabhu et al have prepared first silver NMs by using fermented cow urine which shows antibacterial activities against Gram Positive and Gram-Negative bacterial pathogens. Also confirmed that NMs have anti-phytopathogenic potential against Fusarium Oxysporum NCIM 1008. They reveal that NPs used in cosmetics and wound dressing as nanoweapon to treat fungal and bacterial infection. D.G. Arumugam et al synthesized Panchagavya mediated Copper nanoparticles and study their antioxidant activity by using DPPH (2,2, Diphenyl-1-Picrylhydrazyl) free radical scavenging assay. Also studied cytotoxicity by using brine shrimp lethality assay. P. Chamoli et al prepared Graphene oxide nanoparticles using Hummer′s method, then they reduced it into Graphene nanoparticles using cow urine and urea as reducing agent. They reveals that the clean, green and efficient way of synthesizing graphene using cow urine and urea to produce graphene based transparent conductive films. M. Padvi et al first time successfully synthesized copper oxide nanoparticles by using cow urine and studied antimicrobial activities by Agar Disc Diffusion Method against three pathogens namely Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa. Also observed that increase the effectiveness of the antibiotic tetracycline when synthesized NPs combination with tetracycline.

S. R. Prasad et al successfully synthesized Pd nanoparticles using cow urine as a reducing agent. These synthesized nanoparticles show catalytic efficacy for simple organic transformation. Also studied antimicrobial and antifungal activities. N Jain et al synthesized Silver nanoparticles from Honey and Gomutra by Hydrothermal method and studied antibacterial activity of synthesized silver nanoparticles against Pseudomonas sp. S P Vinay et al synthesized silver oxide (Ag2O) nanoparticles using cow urine by combustion method. They studied Photocatalytic degradation of methylene blue dye and antimicrobial activity. M.K Satheeshkumar et al successfully synthesized copper ferrite nanoparticles by sol-gel auto combustion method using cow urine as a chelating agent. P Chamoli et al synthesized N-doped Graphene Nano-sheet by simple solvo-thermal method using cow urine as a natural dopant of nitrogen. K H Suk et al prepared cow urine encapsulated cellulose nanoparticles and study their antimicrobial activities by using disc diffusion and turbidimetric assays.

Possible Mechanism for Biological Synthesis of Metallic Nanoparticles using Indian cow urine: The researchers have tried to synthesize some transition metal and metal oxide nanoparticles using plant extracts. Various theories have been proposed for the synthesis of nanoparticles using plant extracts. The synthesis of metallic nanoparticles using cow urine is the infant stage. Rai Dhirendra Prasad is a distinguished veterinary scientist from Bihar Veterinary College, Patna and worked as veterinary doctor in government of Maharashtra, India. He theoretically put forward the concept of synthesis of transition metal nanomaterials first time globally. The earlier researchers have tried to propose the possible mechanism for synthesis of materials at nanoscale. (1) Prasad et al have synthesized Palladium and Silver (Prasad 2021) nanoparticles using Indian cow urine. They assume that the urea in the cow urine may get decomposed giving rise to the formation of ammonia. Ammonia contains a lone pair of electrons that can be donated in a chemical reaction. The possible reaction is mentioned below (Prasad 2020). (2) Dabhane et al proposed a little bit different theory. The chemical composition affirms cow urine contains creatinine, uric acids, carbonic acid, cytokine, lactose and leucocytes.

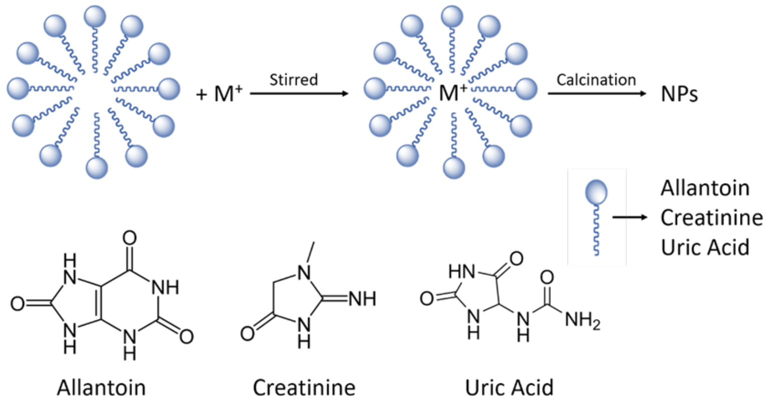

Figure 8. Probable mechanism of green synthesis of Gold NPs using cow urine (Dabhane 2021)

The bio-inspired synthesis of gold nanoparticles using Indian cow urine can be explained as metal ions come in contact with various biomolecules present in cow urine (Dabhane 2021). The Nitrogen containing biomolecules can donate electron and act as natural reducing agent. The plausible reaction mechanism is depicted as below:

7. Conclusion and Perspective

Among various synthetic procedures for the production of nanomaterials, their synthesis using biogenic, natural, non-toxic materials such as cow urine is become crucial area for researchers in nanotechnology. Cow urine have ability to reduce metallic ion into metal nanoparticles. This method is better than physical and chemical method as it uses less hazardous chemicals, require less energy and also economical. In this review, we broadly discussed about nanomaterials which is synthesized by using cow urine such as Silver, Copper, Copper Oxide, Palladium, Graphene etc. Along with synthesis, we studied different characterizations techniques which gives information of size, shape, morphology, stability of the synthesized nanoparticles. Biosynthesized nanomaterials have a potential to explore their uses in biomedical field as compared other area of concerns. Biosynthesized nanomaterials show antimicrobial activity, antibacterial, antineoplastic, Nano catalyst, dye degradation activities. There are also some areas where research has to be done to explore more biomedical applications of nanoparticles and for testing toxicity of synthesized nanoparticles. We know gold nanoparticles have been used in most of the biomedical applications. But there is research about synthesis of gold nanoparticles using biogenic materials. So our area of concern is to synthesize gold nanoparticles using biogenic material such as cow urine and also explore their other biomedical applications.

Author Contributions: All authors contributed equally to the writing of this paper. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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