

NANOPARTICLES AS AN EMERGING TOOL FOR FORENSIC INVESTIGATION

Abstract

Forensic science is the branch of science which uses the application of various branches of science such as Biology, Chemistry, Physics, Biotechnology, Anthropology, Genetics, Nanotechnology, etc. to assist in the examination of various types of evidence to prove or disprove something in front of the court of law. There are varieties of evidence which are found in the crime scene such as biological evidence, toxicological evidence, trace evidence, arson evidence, etc. which has to be examined by a forensic expert. The evidence found in the crime scene is present in very limited or trace amounts hence the forensic expert should ensure to produce accurate results without damaging or destroying the evidences. There are various types of presumptive and confirmatory tests done to find out the identity of the evidence. Some of these tests may damages and destroy the crucial evidence without producing an accurate result. Hence there is a need of such methods from which the analysis of evidence becomes easy in the trace amounts and these tests should produce 100% accurate result. One of these modern methods is the use of nanotechnology in forensic science. Nanotechnology is the technology which uses various types of nanoparticles in the field of science. Nanotechnology includes the use of various types of Nanoparticles for the analysis of various types of evidence found in the crime scene. The Nanoparticles due to their various fascinating properties such as very small size, high stability, large surface-area-to-volume-ratio, interfacial layers, solvent affinity, various mechanical properties, etc. are now-a-days widely in use. Various researchers are continuously researching to find out the more beneficial uses of nanoparticles. This book chapter aims

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to provide an overview of the various uses of nanoparticles in the analysis and examination of the forensic evidences with its benefits over the conventional methods used in forensic science.

Keywords: Forensic Science, Nanoparticles, NPs,

I. INTRODUCTION

Nanoparticles (NPs) are those substances or materials which lie in zero dimension i.e., have dimensions less than 100nm⁽¹⁾. NPs are interesting due to their various characteristics such as melting point, saturation, electrical and thermal conductivity, light absorption, and scattering, these unique properties of NPs make them very useful materials for research resulting in their great performances. NPs are measured in nanometers (nm) and have diameters ranging from 1nm to 100nm⁽²⁾. The International Organization for Standardization (ISO) defines nanomaterials as “Those particles which have external nanoscale dimension or internal nanoscale surface structure are referred as “Nanoparticles”⁽³⁾. It has been found that the size of Gold (Au), Silver (Ag), Platinum (Pt), and Palladium (Pd) can influence the physiochemical properties such as optical properties of any substance, e.g., 20- nm gold (Au), platinum (Pt), silver (Ag), and palladium (Pd). NPs have characteristic wine-red colour, yellowish grey, black and dark black colours, respectively⁽⁴⁾. Au-NPs were synthesized with different sizes and can be further used for bioimaging⁽⁵⁾. NPs are complex molecules and are composed of three layers: The Surface layer, The Shell layer, and The Core layer. The surface layer consists of small molecules, metal ions, surfactants, and polymers. The shell layer has a different chemical composition than the Core. The core is the center and usually consists of the larger area represented in Fig.1. NPs have various applications in Forensic Science.

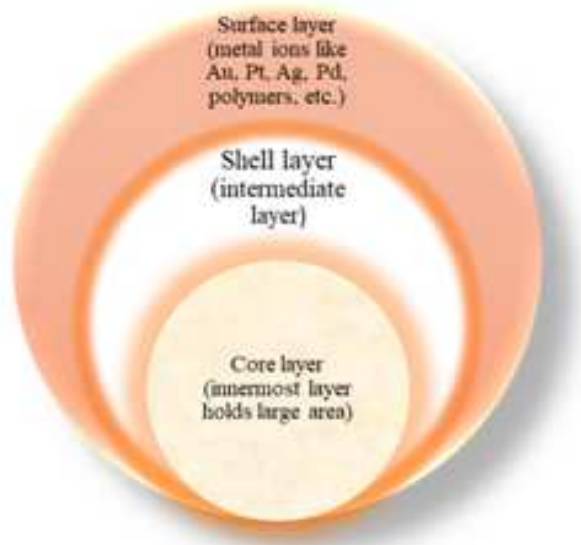


Figure 1: Diagrammatic Representation of Typical Nanoparticle

This chapter mainly focuses on the comparative study of the conventional methods which are used in forensic science to examine various types of evidence such as biological evidence, trace evidence, toxicological evidence, other physical evidence, etc., with the modern methods which use nanotechnology and the nanoparticles in the examination of the various types of evidence found in any crime scene. NPs are widely used in various fields of forensic science such as to identify latent prints, analyze various toxicological samples, to

extract DNA from degraded samples, examine the forged documents, etc. There are various types of NPs which will be discussed in this chapter in later sections.

II. BRIEF HISTORY OF NANOPARTICLES

Metal nanoparticles are able to colour glass in an extraordinary way. Gold has been used for a long time to introduce a striking red colour to glass. One of the finest examples of such ruby glass is the Lycurgus Cup in the British Museum manufactured by Romans in the fourth century it appears with a green colour in daylight, but changes to red, when illuminated from the inside. An interesting fact about the use of gold nanoparticles in ruby glass is that after the Romans the technology was forgotten, and was only rediscovered in Europe in the seventeenth century. Although the birth of gold-based glass and enamel colours is ascribed to Andreas Cassius, who subsequently received the name Purple of Cassius, the preparation of colloidal gold with a tin compound had been described several years earlier by Johann Rudolph Glauber. However, there is no evidence that Glauber ever applied his knowledge to the colouring of glass. It was Johann Kunckel, who ran a glass factory in Potsdam between 1679 and 1689, that successfully used the purple precipitate to produce ruby glass. From a scientific point of view, the next big step forward in nanoparticle research was made by Michael Faraday approximately 150 years ago. As a matter of fact, his systematic studies on the interaction of light with metal nanoparticles can be regarded as the beginning of modern colloid chemistry and the emergence of Nanoscience and Nanotechnology. In the year 1857, he presented his work on 'Experimental Relations of Gold (and other Metals) to Light' to the Royal Society of London⁽⁶⁾. Faraday prepared colloidal gold dispersions in a two-phase system consisting of an aqueous solution of gold salt and a solution of phosphorus in carbon disulfide. After a short reaction time, the bright yellow colour of the Na [AuCl₄] solution turned into a ruby colour characteristic of gold nanoparticles. The principal motivation to perform research on nanoparticles has originated from the so-called quantum size effect. Metal and semiconducting nanoparticles are just a few nanometres in diameter, and thus with sizes somewhere between single atoms/molecules. It shows pronounced size- (and also shape) dependent electronic and optical properties. The observation of such size effects raised expectations for the superior performance of nanomaterials compared to their bulk counterparts in many applications given the size and the shape of the particles can be optimized in a rational way. Systematic work on the photocatalytic properties of colloidal Cadmium Sulphide nanoparticles (Cds-NPs) resulted in the description of the quantum size effect at the beginning of the 1980s. Brus et al. found that Cds-NPs crystallites in the size range of a few nanometres did not have the electronic spectra of the bulk material, even though they exhibited the same unit cell and bond length as the bulk material. These findings opened up a new and exciting possibility to tailor the chemical and physical properties of a material by controlling the crystalline size and shape on a nano-level rather than altering the composition of the materials opening up of plethora of new applicability of these materials. With the advent of technologies now it is possible to control the particle size distribution, shape, and surface properties to apply the nanomaterials in novel ways⁽⁷⁻¹⁰⁾.

III. CLASSIFICATION OF NPs BASED ON THEIR SIZE

In 2000, the first idea of the classification of NPs based on their crystalline form and chemical composition took place⁽¹¹⁾. In 2007, the NPs were classified on the basis of their dimensions such as 0D, 1D, 2D and 3D⁽¹²⁾. This classification depends upon the movement

of electrons in each dimension. Some examples of NPs with different morphologies are Nonporous Pd nanoparticles (0D with 20nm in diameter)^(13,14), Graphene nanosheets (2D) with 1000nm in diameter⁽¹⁵⁾, Ag nanorods (1D) with 100 nm in diameter⁽¹⁶⁾, Polyethylene oxide nanofibers (1D) with 500nm in diameter⁽¹⁷⁾, etc. Other prominent nanoparticles are C60 (1nm), DNA (2nm), UCNP (5-10nm), atoms (0.1nm), Quantum dots (1-5nm), Au nanoparticles (10-25nm), Virus (10-150nm), Ribosome (20nm), etc⁽¹⁸⁾. The various classifications of NPs have been illustrated in Figure 1.

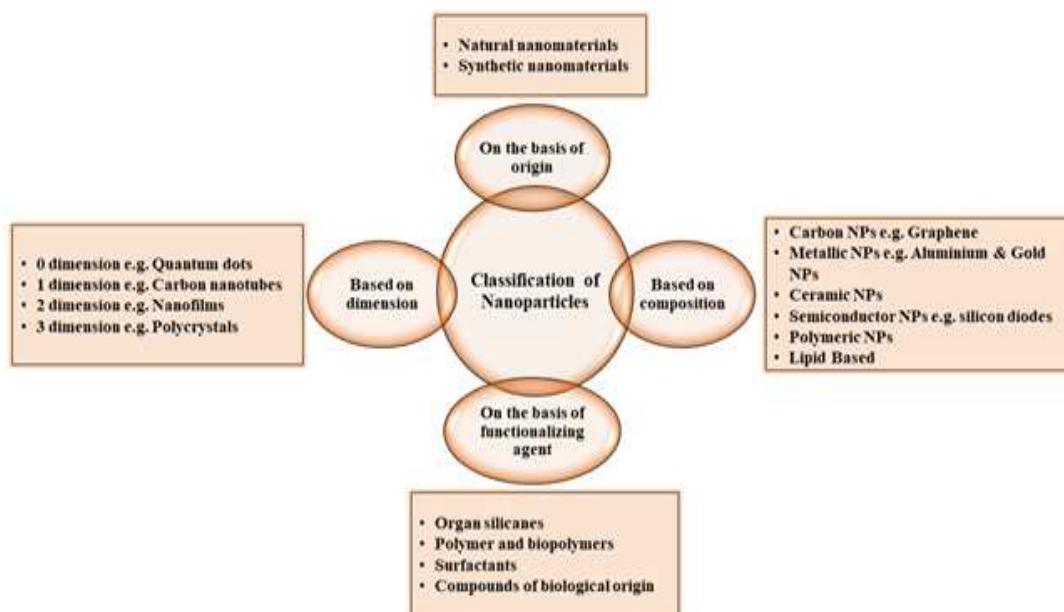


Figure 2: Classification of Nanoparticles

Carbon dots and Quantum dots are the most widely used types of NPs due to their various application-friendly characteristics. Some details of them are as follows:

- 1. Carbon Dots:** Carbon dots (CDs) are nanoparticles or very small particles that lie on zero dimension and show fluorescence characteristics⁽¹⁹⁾. CDs have various properties such as water solubility, biocompatibility, good conductivity, photochemical stability, low toxicity, etc.⁽²⁰⁾. One of the most important application of CDs is targeted drug delivery. When a fluorescent CDs core is attached with a drug moiety, it acts as a drug delivery tool⁽⁴⁾. CDs can be visualized using High-resolution transmission electron microscopy and atomic force microscopy⁽⁸⁾. There are currently two types of CDs: Amorphous based CDs and graphene CDs⁽⁹⁾. The unique photophysical performance of CDs stems from their excitation-wavelength-dependent emission coupled with their enhanced resistance to photo bleaching. Quantum yield of CDs largely depends upon their elemental composition (typically C, H, O, N and, in certain cases, heteroatoms such as S and P), their surface functionalization, and the suspension medium. The pronounced quantum confinement effects have been established for graphene dots, whose cores are composed of a few monolayer's of nanosized graphene. Simple oxidative treatments generate carboxyl and carbonyl groups on the C-dots' surfaces, imparting dispersibility in polar media and securing colloidal stability over a prolonged period of time. In addition, the application of a moderate electrochemical field can induce oxygenated defects as well as

modify the degree of conjugation of the carbogenic core. Frequently described as the nontoxic counterparts of quantum dots, C-dots are synthesized inexpensively by means of pyrolysis or hydrothermal processing of abundant natural resources such as agro-waste and biomass, grass, fruit juice, leaves, glucose, gelatine, eggs, hair fibres, etc. Alternatively, well-defined C-dots can be produced by top-down strategies such as arc discharge, laser ablation, oxidative and electro-oxidative treatment of carbon nanotubes, carbon fibres, activated carbon, exhaust soot, etc. In principle, those methods are scalable and rely on simple synthetic protocols followed by standard purification and size exclusion treatments such as dialysis, centrifugation and filtration. With respect to C-dots' applications, particular emphasis is given to the development of bioimaging nanoprobes with improved spatial resolution and accuracy. Some of the applications of CDs include (i) the development of nano-vehicles for self-targeting drug delivery, (ii) formulation of photodynamic therapy agents, (iii) as antimicrobial materials, (iv) in the development of advanced sensors for chemical and biological compounds, (v) in development of technologies for water and soil decontamination, (vi) in production of slow-release fertilizers, (vii) as a constituent of polymer nanocomposites, (viii) as highly efficient photocatalysts and (ix) as a superior energy convertors⁽⁸⁾.

- 2. Quantum Dots:** Quantum dots (QDs) are those NPs or small-sized artificial semiconductors that show high fluorescence and are used for bioimaging application. The QDs have a core shell structure i.e., the core is inorganic crystalline coated with several ligands suspended in a colloidal solution. When the QDs are alloyed or coated with any alloy from its core to the surface, then it shows continuous fluorescence⁽²¹⁾. QDs can be visualized using a fluorescence microscope as it shows higher fluorescence than fluorescent dyes and proteins. The current discovered QDs are 15-35nm in diameter are elongated in shape and have limited mobility⁽²²⁾. Quantum dots are brightly fluorescent nanocrystals that have found use across a broad spectrum of biological imaging applications. When observed individually under a fluorescence microscope, these particles show a rapid on-and-off 'blinking' of their emission, an attribute that is often detrimental, especially for single-molecule imaging, as the molecules being monitored exhibit frequent loss of signal. In a recent study, it has been reported that quantum dots with an alloyed composition gradient from the core to the surface do not blink but rather remain continuously 'on'. This finding is both surprising and profound, represents considerable progress toward the next generation of fluorescent intracellular probes. Fluorescent dyes and proteins have been invaluable for visualizing the dynamics and interactions of biomolecules within living cells. Unfortunately, light emission by these tags rapidly decays during observation, and the weak intensity of the emitted light cannot be readily detected from single molecules. However, single quantum dots are immensely bright and easily observed using standard fluorescence microscopes, and they emit light hundreds to thousands of times longer than fluorescent dyes and proteins. These two key characteristics have drawn immense interest in the use of these particles for live-cell imaging, but the utility of these particles has been limited, in part, because current commercially available quantum dots (i) rapidly blink, (ii) hydro-dynamically large, (iii) multivalent when functionalized with biological molecules and (iv) often aggregate inside cells.

IV. METHODS OF SYNTHESIS OF NPs

After the discovery of NPs, two approaches were developed for the synthesis of NPs: The Top-Down approach and the Bottom-Up approach.

- 1. Top-Down Approach:** In this method, the bulk or heavy material is broken down to form small sized NPs. This can be done using precision engineering and lithography.
 - **Ball Milling:** John Benjamin developed this method in the year 1970. This method is based upon the reduction of size of any material with high-energy ball milling. In this method, a bulk powder is used with many big-sized balls. Then a high mechanical energy is applied in the bulk powder due to which small-sized nanoparticles are formed ⁽²³⁾
 - **Laser Ablation:** In this method, various materials are converted into nano-sized particles by using laser luminescence. Mainly, a neodymium-doped yttrium aluminium garnet laser is used ⁽²⁴⁾.
 - **Ion Sputtering:** In this method, the spatter of a beam of inert gas ions is used for the process of vaporization of any solid material to form NPs ⁽²⁵⁾.
- 2. Bottom-Up Approach-** In this method, Atom-by-Atom or molecule-by-molecule builds up and forms the NPs ⁽²⁶⁾.

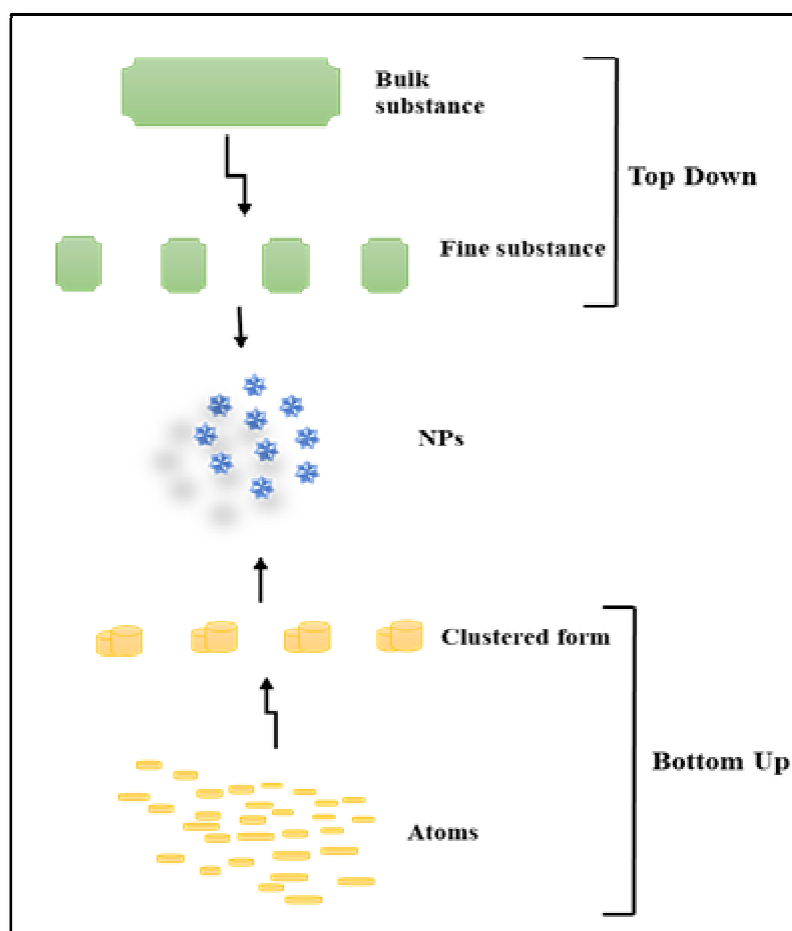


Figure 3: Illustration of synthesizing approaches for nanomaterials

- Physical vapor deposition method- In this method, the materials or the particles get deposited physically on a surface containing thin film and build up to form NPs⁽²⁷⁾.
- Chemical vapor deposition method- In this method, chemical reactions of gaseous molecule containing atoms is performed on the target thin film for the formation of NPs⁽²⁸⁾.
- Sol-gel method- This method can be done in two ways, either by direct mixing of preformed colloids metal (oxide) with a sol containing the matrix-forming species followed by gel formation or mixing of metal or metal oxide directly within a pre-hydrolyzed silica sol⁽²⁹⁾.
- Hydrothermal method- In this method, the reaction of the solid material is done with aqueous solution vapors, under high temperature and pressure. This leads to the formation of the NPs⁽³⁰⁾.

3. Other Synthesis Methods

- **Solvent Extraction/Evaporation:** The organic polymer NPs are fabricated by a solution in Dichloro-methane which is a solvent, followed by sonication, evaporation, filtration, and freeze-drying⁽³¹⁾.
- **Self-Assembly:** The fibrous atoms or molecules arrange themselves in various physical and chemical conditions such as pH, temperature, and solute concentration^(32,33).
- **Layer by Layer Deposition:** The bilayer membrane platforms are fabricated by layering of sodium silicate on gold and calcination is done in the furnace⁽³⁴⁾.
- **Microbial Synthesis:** Some living cells, such as *Aspergillus fumigatus* is used to produce silver NPs extracellularly⁽³⁵⁾.
- **Biomass Reactions:** Dead oat stalks were incubated with an acidic aqueous solution of gold ions to produce gold nanorods and NPs⁽³⁶⁾. Table 1 shows the various methods of synthesis of different NPs which are used in multiple forensic applications.

Table 2: The synthesis methodologies of various NPs used in multiple applications

S No.	Forensic application	Synthesis method	Salts used	Solvent used	Properties	Remarks	Reference
1	Protein purification	Co-precipitation method	FeCl ₂ ·4H ₂ O FeCl ₃ ·6H ₂ O	Distilled water	Size: 10–20 nm	To immobilize several functional proteins without affecting their property.	(152)
2	Nucleic acid analysis	Precipitation method	FeSO ₄ ·7H ₂ O FeCl ₃	Distilled water	Size: 51nm	To perform the readings which confirm the	(153)

						presence of DNA.	
3	Pathogen detection	Hydrothermal reaction method	$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	Glucose and oleic acid	Size: 60nm	To identify and capture variety of pathogens.	(154)
4	Illicit drugs	Commercially available magnetic NPs	-	-	Core size: 50-20 nm shell structure: 2-5 nm thickness surface roughness -36.63 nm	It is a rapid lateral flow immunoassay which does not require any sample pretreatment.	(155)
5	Anti-counterfeiting and security applications	High-temperature hydrolysis process	FeCl_3	DEG	Size: 30-180 nm	Instant response to magnetic field of colloidal nanocrystals structure.	(156)
6	Arson agent detection	Commercially available magnetic NPs	Fe_2CO_3	Acetonitrile	Size: 66-70 nm	To extract the markers, present in the warfare organic liquids	(157)
7	Explosive detection	Solvothermal method	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	Ethylene glycol	Diameter-400nm, Shell thickness-15nm	These are used to detect the presence of trinitrophenol	(158)

V. APPLICATIONS OF NPs

Table 3: Applications of Nanoparticles and their properties

S. No.	Nanoparticles(NPs)Type	Significance	Properties
1.	Inorganic NPs Also known as ceramics NPs such as gold NPs, mesoporous silica	Drugs and medication, food packaging, drug	Increases therapeutic properties of drug, Non-toxic, hydrophilic, highly stable,

	NPs, Quantum dots etc.	delivery, etc.	photocatalysis, image applications etc.
2.	Metallic Nanoparticles such as Ti, Cu, Zn, Ag, Au, etc.	Biosensors such as glucose Nano sensors, lactate nano sensors, urea nano sensors etc. used as cutting-edge materials, Au NPs coating is used in the sampling of SEM etc.	Unique optoelectrical properties Due to well-known localized surface plasmon resonance (LSPR), Sensitivity, selectivity, linearity, stability, response time, and reproducibility.
3.	Micelles, liposomes, nano emulsions, Biopolymeric NPs, cubosomes etc.	Used in food industry	Promotes sensitive components in the food from degradation, increases the bioavailability of micronutrients like iron, promotes the efficacy of food additives, and increases shelf life of meat.
4.	TiO ₂ NPs, SiO ₂ NPs	Dye and paint industries	Various chemical resistance properties, Thermal stability, easy to clean, scratch resistance, good mechanical and electrical properties, etc.
5.	Carbon-based NPs such as fullerene, Carbon nano tubes etc.	Used as fillers, efficient gas adsorbents, environmental remediation etc.	High specific surface area, high carrier mobility, high electrical conductivity, flexibility, optical transparency, etc.
6.	Semiconductor NPs such as CdSe NPs, CdS NPs, ZnS NPs, ZnO NPs etc.	Used in optical devices, photo optics, electronic devices, water-splitting applications etc.	They possess wide bandgaps and hence show significant alterations in their properties with bandgap tuning.
7.	Lipid-based NPs such as lipid NPs etc.	Biomedical applications, drug carriers and delivery, RNA release in cancer therapy etc.	They are spherical with diameters ranging from 10-1000 nm.

VI. ADVANTAGES OF NPs OVER CONVENTIONAL METHODS USED IN FORENSIC SCIENCE

Conventional methods are those methods that are used for a long period of time. The cyanoacrylate fuming method, silver nitrate method, ninhydrin method, etc. are various conventional chemical methods that are used for the development of latent fingerprints. These chemical methods have various disadvantages. The cyanoacrylate method is hazardous to health and has various security issues. In the silver nitrate method, the fluorescence of silver decreases over time, similarly, the ninhydrin method shows less contrast and is less sensitive^(37,38). To overcome this, Nanophosphors are used for the detection of latent fingerprints. Nanophosphors show an important property of UV radiation-dependent photoluminescence emission and improved adhesive property, due to which it is more accurate. These nanophosphors can be used in both porous and non-porous surfaces. The latent prints that are treated with nano phosphors can be preserved for a longer time as it does not lose its photoluminescence property⁽³⁹⁾.

In the cases of extraction of the DNA from the degraded bones, skeletal remains, blood, saliva, semen, hair, urine, and other biological samples small size loci amplification by polymerase chain reaction is used⁽⁴⁰⁾. This DNA extraction method is very complicated, time-consuming, and involves a lot of steps such as centrifugation, precipitation, etc.⁽⁴¹⁾. Therefore, magnetic NPs are used for the faster DNA extraction process. These NPs are highly rapid, cost-effective, less time-consuming, and have a unique size and various physical properties⁽⁴²⁾. The integrated systems of magnetic NPs such as copper NPs are developed which consist of solid phase systems has the advantage of minimum DNA degradation and maximum DNA yield^(43, 44). The above-mentioned examples of conventional methods are widely used for the analysis of the evidence but are inaccurate, time-consuming, hazardous etc., hence there is a need to initiate the use of NPs based examination of the evidence to produce accurate and appropriate results. Following sections will highlight the applications of NPs in various fields of Forensic investigations.

VII. APPLICATIONS OF NPs IN FORENSIC SCIENCE

The Application of nanoparticles is increasing day by day on an exponential manner in every walk of life. Some important and crucial applications of NPs are listed in Table 3. The field of forensic science is growing rapidly. Forensic investigations include the development of new techniques such as NPs based techniques that helped in investigations.

- 1. Significance of NPs in Biological Evidences:** In any crime scene, various types of evidence can be found such as biological evidences, trace evidences, etc. Therefore, to identify the suspect and to examine the cause of death, the examination of biological materials is an important task. In the field of Forensic Science, magnetic NPs got greater attention as they have a large surface area, superparamagnetic properties, stable physical properties etc.⁽⁴⁵⁻⁴⁷⁾.
 - **Separation of Biomolecules and Cells:** Magnetic NPs play a vital role in the separation of nucleic acids, proteins and cells due to its magnetic separation and diversity of surface modification properties.

- **Nucleic Acids:** DNA plays a vital role in the investigation process and the establishment of the evidence with the suspect. Genetic markers analysis such as short tandem repeats, single nucleotide polymorphism, and insertion-deletion polymorphism can be used to identify the suspect and used for the suspect individualization⁽⁴⁸⁾. The phenol-chloroform is a conventional method used by forensic experts to extract the DNA from the cellular membrane. This method is a very time-consuming method and involves processes like incubation, centrifugation, and a lot of chemical preparation. The Chelex resin is another method of DNA extraction in which resins of styrene divinyl benzene copolymers is used containing paired iminodiacetate ions which chelate metal ions. This method involves heating at a high temperature due to which single stranded DNA is yielded, hence it can't be used to perform downstream process such as RFLP (Restriction fragment length polymorphism) and PCR (polymerase chain reaction) can be performed only⁽⁴⁹⁾. Hence, as compared to these conventional methods, the Magnetic Solid Phase Extraction (MSPE) of the DNA is widely used to extract DNA sample from the biological evidence. The MSPE does not use any toxic chemicals as compared to phenol-chloroform extraction and simplifies the sample preparation procedure⁽⁵⁰⁾. MSPE is performed without doing centrifugation which reduces the risk of contamination while separating the DNA. In MSPE, the silica-based materials are selected as the solid phase or the shell layer as it has the property to distribute the charge in all over its surface area. As the pure silica is negatively charged, it does not absorb the DNA due to electrostatic repulsion. Hence, to enhance the affinity between the silica layer and the DNA molecule, various chaotropic agents such as GuHCl and NaClO₄ are added into the solid phase consisting of silica⁽⁵¹⁾. The DNA strand will stick to the solid phase and all the other unnecessary products were removed by washing with ethanol. Then elution of DNA is done in a low ionic strength buffer. To increase the effectiveness of this method, mesoporous silica is developed into the magnetic pore to increase the adsorption capacity of the DNA. The mesoporous silica contains ordered pore and ultra-high specific pore volume that allows the uniform and effective binding of the DNA⁽⁵²⁾. The chaotropic agents also have some disadvantages, like it has the capacity to degenerate the DNA fragments. Hence, to avoid the use of chaotropic agents, amino groups are added into the mesoporous silica magnetic NPs which results in much higher and more efficient DNA binding capability⁽⁵³⁾. The mesoporous silica-coated magnetic NPs have an equilibrium adsorption time of 10 hours to adsorb DNA⁽⁵⁴⁾. Some positively charged polymers such as polyaniline, polyaniline-chitosan, and polyethyleneimine have been used to shorten the equilibrium adsorption time of the DNA^(55,56,57). An experiment shows that the polyaniline-modified γ -Fe₂O₃ magnetic NPs shows an equilibrium adsorption time of only 10min⁽⁵⁶⁾. Some magnetic NPs have also been developed which have high adsorption capacity and short equilibrium adsorption time to produce high-yield DNA from various biological samples⁽⁵⁸⁾. It is also found that MSPE can also extract DNA from decomposed samples such as waterlogged bones in drowning cases⁽⁵⁹⁾, samples which is found in PCR inhibitor containing substrate like blood in soil with humic acid⁽⁶⁰⁾, etc. Hence it can be concluded that MSPE can be used in various forensic laboratories to extract the DNA from various types of biological samples found at a crime scene.

- **Mixture of Cell:** In cases of sexual assault such as rape, the biological sample which is collected will be a mixture of the female's vaginal secretion and the male's semen. The semen is found at a low level as compared to the vaginal secretion. In order to perform the genotyping of the semen sample, the following three steps are done. (i) Separation of sperm cells from the mixture of epithelial cells of vagina and semen before extraction, (ii) Selection of genetic marker during PCR, and (iii) Analysis of result after DNA typing from the sample. To separate the sperm cells from the vaginal epithelial cells, Magnetic Activated Cell Sorting (MACS) based on MPSE is used. In an experiment, Anslinger et al. coupled the angiotensin-converting enzyme (ACE) antibodies with Magnetic NPs by using the Pan Mouse IgG antibody as a coupling bridge⁽⁶¹⁾. These monoclonal antibodies show high anti-sperm specificity towards ACE, hence it can be used to separate the sperm from the mixture. This method is only suitable for enough quantity of sperm containing flagella which have the presence of ACE. This creates a problem as the older vaginal swabs usually lack flagella. Therefore, Li et al. stated that magnetic NPs coupled with antibodies against motile sperm domain-containing protein 3 (MOSPD3) can efficiently extract the DNA from the sperm cells (older with no flagella) as it is found that MOSPD3 is most abundant in sperm head and neck⁽⁶²⁾. To make this process more efficient, Zhao et al. covalently immobilized anti-sperm adhesion molecule 1 (SPAM1) onto Magnetic NPs. SPAM1 which is a hyaluronidase presented on the head of sperm⁽⁶²⁾. In the case of gang rape, a mixture of semen is found having various sperm cells; hence individualization will be a difficult task. Xu et al. utilized Magnetic NP-bound, A-kinase anchor protein 3 (AKAP3) antibodies to extract different sperm cells present in the vaginal fluid. He then used ABO blood-type antigen antibodies based on flow cytometry to extract sperm cells of individual males⁽⁶³⁾. This sorting strategy based on ABO has also a limitation that the sperm cells from the same secretory ABO can't be isolated.

- **Diatoms:** Diatom is a widely occurring algae found in water bodies, are the cells of great interest in forensic science. Diatoms are used in cases of drowning, mainly to find out the antemortem or postmortem death. The nature i.e., antemortem or postmortem can be found out from the diatoms present in the cadaveric tissues of the corpse⁽⁶⁴⁾. The cell wall and frustule of the diatom is made up of silica which shows a high DNA binding property in the presence of chaotropic agents. In a recent experiment, α DNA-coated Magnetic NPs were applied as sorbents to separate diatoms from foreign impurities coming from routine acid digested tissue⁽⁶⁵⁾. The presence of diatoms can be visually seen in the microscope.

- **Examination of Creatinine in Urine:** Creatinine is a metabolic biproduct of kidney which acts as an indicator for renal functions. The creatinine is produced continuously in the kidney and is excreted into the urine by glomerulus⁽⁶⁶⁾. The levels of creatinine are indicated by glomerular filtration rate (GFR) and the normal GFR range is 90-120 mL /min. Patients of kidney related disease have GFR range of 15ml /min⁽⁶⁷⁾. The creatinine level of the urine sample can help us to link with the creatinine level of the suspect by measuring the quantity of creatinine. Various conventional methods such as Jaffe's color test for creatinine, high-performance liquid chromatography (HPLC), liquid chromatography–tandem mass spectrometry (LC–MS/ MS), enzymatic-based sensing, and nonenzymatic electro-chemical-based sensing are used for the detection

of creatinine. In Jaffe's test, oxidation reaction takes place in the presence of picric acid, which forms a coloured compound of creatinine picrate. LC-MS/MS uses stable isotope dilution to detect the presence of creatinine. These conventional methods are time consuming and is not efficient and accurate. In some cases, sodium dodecyl sulphate was added to remove the bilirubin from the urine sample to remove the interference⁽⁶⁸⁾. To overcome these, the nonenzymatic electrochemical methods are used. polyethyleneimine with phosphor tungstic-acid multilayer modified electrode is used which shows much more accurate result for the creatinine⁽⁶⁹⁾.

- **Examination of Blood Samples:** Blood is the most common and crucial biological evidence found at any crime scene and the analysis of blood stains found in any crime scene is a great source of information for the forensic examiners⁽⁷⁰⁾. The prime role after finding any stain at any crime scene is to confirm the stain as blood. This requires some physical examination followed by presumptive tests. Some presumptive tests of blood include Kastle Mayer test, Tetramethyl Benzidine test etc. These are the colour tests which is based upon the oxidation reaction catalyse by the heme molecule. These tests use hazardous chemicals. At almost every crime scene, Investigator get very limited amount of blood hence, Investigator have to rely upon those techniques which requires very less amount of sample and is non-destructive technique. Apart that, technique should be more specific, rapid, highly sensitive, along with portable, and easy-to-use instrumentation.

For the identification of human body fluids (mainly blood) present in trace amount at any crime scene in dried form, a recently developed very effective technique used is Surface enhanced Raman spectroscopy (SERS) in which Au and Ag nanostructured substrates excitation done at 785 nm⁽⁷¹⁾. Recently, developed SERS confirmatory identification technique can detect the dried blood upto 105 times dilution by using Au-NPs substrates. The SERS spectral lines can be observed within a minute due to the presence of heme prosthetic group of the haemoglobin protein by this method. In this process, acetic acid is used as it has the ability to denature the haemoglobin and it allows the heme moiety to absorb into the metal surface efficiently. There will be a completely different spectra if we will use Ag in place of Au metal as the charge transfer characters will change upon change in the metal⁽⁷²⁾.

To differentiation between the menstrual blood with the peripheral blood is a cumbersome and challenging on evidence analysis. This can be overcome by SERS using Au NPs substrates, which will give different type of spectra lines for menstrual and peripheral blood. This can be examined by observing the spectral lines and compare it with the statistical data to achieve a successful classification⁽⁷³⁾.

- **Examination of Uric Acid in Blood Serum and Urine:** Uric Acid (UA) is an important biomolecule which is produced as a result of final product of degradation of purines nucleotides, which is found in blood serum and urine⁽⁷⁴⁾. The amount of UA is the marker for various diseases such as hyperuricemia, renal disease, gout etc. There are various methods for the examination of UA such as fluorescence spectroscopy, capillary electrophoresis, high performance liquid chromatography and biozymatic colorimetry. These methods are highly accurate and sensitive but also has various drawbacks. They require various types of complex equipment's, sample

preparation, and takes very long time for the analysis. The cost of these equipment is very high and cannot be used for on-site analysis.

To overcome these drawbacks, electrochemical sensing is used for the analysis of UA. The electrochemical sensing is simple, inexpensive, fast, highly sensitive and suitable technique for on-site monitoring^(75,76). Various types of non-enzymatic sensors are also developed that uses various types of transitional metal NPs such as Pd, Pt and Au as the catalytic materials⁽⁷⁷⁾. These NPs have large surface area, shows good conductivity and stability, have small dimensional size and excellent catalytic properties⁽⁷⁸⁾. To increase the electrocatalytic performance and sensitivity of sensors, the AuNPs are used in the combination with carbon NPs, such as mesoporous carbon⁽⁷⁹⁾.

- 2. Significance of NPs in Explosive Evidences:** Terrorism and mass destruction in the society creates a feeling of terror in the citizens of any nation. The act of terrorism takes place by using various kinds of explosives. In some cases, very less amount of unexploded explosive or the contaminated sample is found. In these type of cases, use of nanotechnology for the identification traces of explosive materials can be done⁽⁸⁰⁾. According to Pandya and Shukla 2018, Turmeric extracted curcumin nanoparticles-based, which are highly selective, and ultrasensitive fluorescent probe is used to detect Trinitrotoluene (TNT) up to 1nm level in aqueous solution. As these nanomaterials have high surface area-to-volume ratio, these can be used to detect the presence the traces of explosive materials, explosive materials in vapour form etc.

To achieve this, nanomaterials-based sensors are used as they are of low cost, high sensitivity, low consumption of power, and advanced stability. In these types of sensors, various nanomaterials and nanodevices such as nanobots, electronic noses, nano-mechanical devices, nanofabricated structures, nanotubes, and nanowires are widely used. The nano sensors can detect Research Department explosive(RDX), TNT, DNT (2,4-dinitrotoluene), Pentaerythritol tetranitrate (PETN), Ammonium nitrate and High Melting Explosives (HMX). In 2014, Ma et al. states that, in some cases, for the detection of DNT, single-crystalline semiconductor CdS nano slabs were used on a silver surface. The detection of explosives can also be achieved by Surface enhanced Raman spectroscopy/scattering (SERS) using gold NPs substrate which provides strong and specific binding of molecules with the surface. The Nitroaromatic explosives shows great affinity with gold NPs as the nitro group forms the bidentate chelate with the oxygen atoms present in its surface. Hence, SERS is used for the detection of nitroaromatic explosives in the vapor form⁽⁸¹⁾. Triacetotriperoxide (TATP) is an explosive that lacks nitro group, but can be detected using gold NPs in variable temperatures⁽⁸²⁾. The silver-based NPs reduced on silica films can also be used for the detection of TNT, PETN, RDX and TNB⁽⁸³⁾. Luminescence based detection of the explosive substance is another method for the detection of explosive found in any crime scene. This technique offers very highly efficient detection of the explosive material under low or zero background with high power source. This test is usually performed to detect the presence of TNT and DNT. In these techniques, the explosives are manufactured containing reporters' dye or any fluorescent dye, which has the highly luminescence property, but these dyes have various drawback such as limited quantum yield, high toxicity, stability and labelling efficiency. To overcome these, use of NPs such as non-metal, metal and metal oxide NPs, QDs, NPs

based on carbon, lanthanide doping and fluorescent dye, metal organic frameworks etc has been initiated for the detection of explosive ⁽⁸⁴⁾. The figure 3 shows application of NPs in explosive detection.

- 3. Significance of NPs in Arson Evidences:** The Fire debris detection and analysis are composite tasks in arson cases. This is a challenging task due to the very high temperatures and high destruction caused due to arson. This arson can be caused due to presence of various accelerants like petrol, diesel, kerosene, etc. These accelerators can be found in the crime scene in the form of vapor, traces on floor, door, roof, etc., or in any container found at the crime scene. These fire residues can be analyzed using passive absorption and concentration of the vapors on charcoal and analysis by gas chromatography.

The use of SERS for the characterization of gasoline or other ignitable liquids in arson has been widely seen ⁽⁸⁵⁾. SERS is also used for the analysis of polycyclic aromatic hydrocarbons (PAH) ⁽⁸⁶⁾. Besides that, is the use of cutting-edge nano based analytical tools for the process of detecting the arson agents is proving to be very helpful in Forensic investigations. The arson or the warfare agents are divided in to two types based upon their action in human body. These are Chemical warfare agents (CWA) and biological warfare agents (BWA) ⁽⁸⁷⁾. Singh et al. gave a method to identify CWA using magnetic dispersive solid phase extraction (MDSPE) followed by gas chromatography mass spectroscopy analysis ⁽⁸⁸⁾. In this method, Iron Oxide (Fe_2O_3) @ Poly (meth-acrylic acid-co-ethylene glycol Di methacrylate) resin (Fe_2O_3 @ Poly (MAA-co-EGDMA)) is used as a sorbent. This method is useful for offsite examination of CWA. In addition to this a method has been developed by which Magnetic NPs peroxidase mimetic based colorimetric method ⁽⁸⁹⁾. R. Khak-Sarnejad suggested (Silicon Oxide)that SiO_2 -magnetic NPs based biosensors can be used for the detection of the organophosphorus compounds used as nerve agents (NA) and CWA ⁽⁹⁰⁾. Another method implies the use of Star-shaped AgFeO_2 @Au/Ag NPs based SERS substrate which is used for the detection of paraoxon ethyl, which is a CWA molecule ⁽⁹¹⁾. Pal et al. gave a method of identification of BWA in which he suggested the use of electrically active (EA) magnetic NPs. This magnetically active NPs work as a direct charge transfer biosensor to detect *Bacillus anthracis* Sterne endospores ⁽⁹²⁾.

- 4. Significance of NPs In Gun Shot Residues:** Gunshot residues (GSR) are those particulate matter or residues that are found as the by product of firing. The GSR is a residual discharge from a firearm and plays a vital role as evidence in investigations. These GSRs can be organic or inorganic which can be recovered from the hands of the suspect, clothes, near the wound, in the crime scene etc. The GSR examination mainly focuses on the quantitative detection of the heavy metals i.e., barium, lead, antimony etc. and characterization is done which is carried out by using Scanning Electron Microscopy (SEM) with Energy Dispersive Spectroscopy (EDS) i.e., SEM/EDS ⁽⁹³⁾. There are various conventional methods for the analysis of GSR which is bases upon the detection of the presence of nitrites and nitrates in the GSR. These conventional methods are not accurate and is time consuming. Hence, NPs such as gold NPs are used for the analysis of GSR more accurately ⁽⁹⁴⁾. The ultra-sensitive nano sensors play an important role in the detection of the GSR as it exhibits the property of high surface-area-to-volume ratio and quantum confinement. These ultra-sensitive nano sensors can sense up to micrograms of

the sample ⁽⁹⁵⁾. A recent development of electrochemical sensors based on the chemical alteration of electrodes using Nobel atom particles such as platinum (Pt), gold (Au), silver (Ag) and palladium (Pd) on the nano and microscale level is done. These atom increases the active surface area and the electrocatalytic properties of the electrode ⁽⁹⁶⁾. The catalytic property of the palladium can also be increased by synthesizing the Pd in carbon nanotubes, graphene and carbon microspheres. The Calcium Oxide (CaO) NPs are also used in the examination of GSR to determine whether the shooting is done with the intention to kill the person or is done as an act of self-defence. The GSR analysis involves the rearrangement of the GSR particles using High Resolution Scanning Electron Microscopy (HR-SEM) images ⁽⁹⁸⁾. To determine the presence of chemicals in GSR X-ray spectrometer in conjunction with a scanning electron microscope is used.

5. Significance of NPs in Analysis of Toxicological Evidences

- **Examination of Illicit Drugs:** Illicit drugs or prohibited drugs, especially narcotics are a wide area of concern for forensic examiners as these drugs are exported, imported, or used without any legal permission. The drugs trafficking has increased at an alarming rate hence, its examination is an important and challenging task. There are various color tests which are used for the preliminary examination of drugs such as Scott Test for Cocaine, Marke's test for Heroine, Dilli kopenoys test for barbiturates etc. These color tests are not accurate and some color tests produces same color for two different drugs. The confirmatory tests are based upon the instrumental examinations which requires a lot of time and complicated instrumentation setup, the use of magnetic NPs can overcome these drawbacks. To improve or to modify the detection technique of drugs, Sanli et al. has developed a biosensor coated with antibody functionalized magnetic NPs to detect the presence of synthetic cannabinoids in the Urine sample. In this method, the iron oxide NPs are manufactured by co precipitation method, and then the amino groups are functionalized by using Stöber's method. Then the immobilization of the functionalizes magnetic NPs is done by monoclonal anti-K2 antibody. This biosensor works on the principle of differential pulse voltammetry technique with a potential range from -0.4 to $+0.8$ Volt. This method is widely used to detect the concentration of cocaine in the urine. The pulse signals of the biosensor will increase gradually upon increase in the amount of cocaine in the sample. During the autopsy, the blood sample should be collected and analysis of drugs in that blood sample should be done as in most of the cases, the drug is administered intravenously i.e., via the veins. In some cases, such as chronic arson poisoning, the hair samples can give the best results hence these should also be preserved. To identify the presence of drugs in these biological samples, Boojaria et al. uses saline modified magnetic NPs to detect the presence of morphine (mainly in hair sample). In this method, he first removed the contamination by treating the hair sample with dichloromethane and methanol solvent. Then he divided and cut the hair in proper dimension. In the final stage he used methanol solvent for the digestion of the hair sample. The main principle of this test is to perform the quantitative analysis of the morphine by using magnetic NPs based solid state extraction in association with liquid chromatography technique coupled with diode array detection. This method also detects the trace amount of drug in the sample and quantify the morphine ⁽⁹⁸⁾.

Shiri et al. gave another method of examination and quantification in which the magnetic NPs are used as efficient adsorbents to extract different drugs such as Fenopropfen calcium (FPC), methocarbamol (MTC), clonazepam (CZP), and ibuprofen (IFB) in magnetic dispersive solid-phase extraction (MDSPE). In this method, the synthesized magnetic NPs were spread into the solution containing the drug sample and later the magnetic NPs were separated using external magnetic fields. The magnetic NPs were eluted using methanol and the elution was analysed using High-performance liquid chromatography (HPLC). This technique will quantify the drugs in human serum and urine^(100,101).

Another method involves the use of functionalized NPs such as Graphene oxide, CNT electrodes⁽¹⁰²⁾, Au Nanoprobes⁽¹⁰³⁾ etc. which can differentiate very less quantities of any drug as they show high electrical conductivity and sensitivity⁽¹⁰⁴⁾. Semiconductor NPs and QDs have also been used on the fluorescence-based detection of the drugs as they are very stable and exhibit great fluorescence properties.

- **Examination of Clinical Medicines:** Clinical medicines or drugs are those medicines which are used for various clinical purposes and these medicines are not prohibited by the government. These medicines are also widely misused as people get addicted to it as it gives them relief and pleasure. The high dosage of these medicines can also be fatal; hence a particular dose of each medicine has been decided before its launch. These medicines have been misused by various ways. Some medicines are remade as they are quite expensive. So various chemicals are added which mimics the property of that medicine but that medicine fails the authenticity and purity test and can be highly dangerous. These types of medicines are sold illegally. Studies found that these medicines are very prone to Hypotension and Syncope. Hence, the detection of these types of drugs will be a necessary task. To perform the task, Magnetic NPs can be widely used. These magnetic NPs have various appreciable properties such as dispersibility, large surface area, surface tunability etc.

Botulinum neurotoxin (BoNT) is a type of toxin, which is used as a clinical medicine in BOTOX® and Dysport® cosmetics⁽¹⁰⁵⁾. The overdose of these medicines will cause a severe illness known as Botulism. To detect the amount of these medicines i.e., BoNT types A, B, and E in blood, Orlov et al. Multiplex lateral flow (LF) assay⁽¹⁰⁶⁾. In this method, Magnetic NPs-antibody (AB) conjugate is used. This assay is based on the sandwich mechanism of lateral flow in which the sample is loaded into sample well and migration of the sample takes place according to the capillary action. Due to the capillary action the sample comes in contact with the magnetic NPs-AB conjugate. If the target antigen will be present in the sample, then the antigen will bind with NPs-AB conjugate and capture the AB present on the test line. These testing are done in LF test strips. There are 3 test strips for A, B and E type⁽¹⁰⁷⁾. The magnetic NPs can facilitate the quantitative detection of abuse clinical drugs. Hence magnetic NPs should be used in the forensic drug detection.

6. **Significance of NPs in Questioned Document Examination:** Questioned documents are those documents whose authenticity is under question in front of law. The examination of the questioned document is an important task for the questioned document examiner to prove its authenticity for judicial purposes. Advanced techniques such as

fluorescent/optical inks, security fibres, and planchettes have also been developed to prevent counterfeiting⁽¹⁰⁸⁾. These methods are although accurate at some level, but still compromised to prevent losses and counterfeiting. The phosphorous used in fluorescent techniques have a disadvantage i.e., its size will decrease rapidly while the process undergoes. Hence it will affect its luminescence property. Therefore, these methods are not much effective and it reduces the security feature efficiency⁽¹⁰⁹⁾. To overcome the limitations, Photonic crystals (PC) are developed⁽¹¹⁰⁾. The PC are the periodic dielectric structure which forms the photon energy band and allows the propagation of Electromagnetic waves having specific frequency^(111,112). The magnetic PCs show photonic properties which are produced due to the embedment of magnetic NPs in the building blocks⁽¹¹³⁾. Ge et al. prepared polyacrylate-capped superparamagnetic magnetite (Fe₃O₄) colloidal nanocrystal clusters (CNCs) with tuneable stop bands covering the entire visible spectrum having rapid and fully reversible optical response within 200 Ms after application of the magnetic field⁽¹¹⁴⁾. Further Kim et al. worked on magnetically tuneable and lithographically fixable PC to produce structural color printing⁽¹¹⁴⁾. This method shows the use of a single M-ink containing an assembly of super magnetic CNC in the polymer synthesized from poly (ethylene glycol) diacrylate (PEG-DA) and 2,2-dimethoxy-2-phenyl acetophenone as a photo initiator to produce a variety of multi-coloured structural patterns. These M-ink can be used in currency notes, control the forgery of sensitive documents, and produce a variety of art materials with unique patterns. Another counterfeiting ink has also been developed which uses spirogyra into copolymer latex nanoparticles chemically based on methyl methacrylate and butyl acrylate by the method of semi-continuous mini emulsion polymerization. By this a high security, invisible ink has been created which can be used in the important documents such as passports, certificates as well as in bank notes as anticounterfeiting ink. When these documents are exposed under UV radiation, they show photochromism i.e., changes its colour from colourless to purple and shows red fluorescence⁽¹¹⁵⁾. For the detection of inks is based upon Surface Assisted Laser Desorption/Ionization Mass Spectroscopy (SALDI-MS) in which carbon NPs, Metallic NPs, and silicon NPs have been utilized. This helps in the detection of inks and visible dyes on bank notes and question documents. It also helps in the identification of ink strokes for tracing forgery and other alterations⁽¹¹⁶⁾. Another microscopy technique is atomic force microscopy which is also used for the identification of the sequence of pen strokes, amplitude and phase change of ink on paper, ink crossing etc⁽¹¹⁷⁾.

In a study, nitrogen-doped CDs (N-CDs) has excellent thermal 100 degrees Celsius and no loss in its fluorescence and photostability performance, shows illumination in UV and visible region, has high quantum yield etc. The fluorescence property of the N-CDs will gradually increase upon an increase in temperature. The N-CDs are widely used as an invisible ink for imprinting the secret information in any document to prevent it from counterfeiting⁽¹¹⁸⁾. For the purpose of visualization of these CDs in any document, Polyvinyl alcohol (PVA) is used. The N-CDs/PVA complex composite film gives a bright blue fluorescence when observed under UV radiation. Also, it shows 90% transmittance in the visible region. Fernandes et. al demonstrated the use of carbogenically coated silica NPs (C-SiO₂) for the purpose of nano tagging to mark and authenticate products. The C-SiO₂ is used for the process for solvent evaporation in which the C-SiO₂ aggregates spontaneously and generates nonduplicable photoluminescent motives. When tagging with C-SiO₂ will be done in any document, it will show high degree of fluorescence when

observed under UV light ⁽¹¹⁹⁾. Under the examination of the questioned document, the foremost important task is the analysis of ink present on the questioned document. There are mainly three pen ink groups i.e., Fountain pens which use water based liquid ink, Ball point pen which use viscous oil-based inks and Roller ball pens or gel pens which use water-based liquid or gel ink. On the other hand, various types of printer inks include Toner based printers, Liquid inkjet printers, Solid ink printers, Dye sublimation printers, Inkless printers, etc. For the analysis of inks, various types of chromatography methods such as Thin Layer Chromatography and various spectroscopic methods are used. These methods are destructive methods, which damages the document and are not accurate. The analysis of ink in the questioned document is done in two phases, (i) determining the age of the document by performing dating of the ink, (ii) the analysis of ink composition to identify the ink or compare the ink and its strokes. As the ink contains various volatile compounds i.e., solvents, which are volatile in nature will start evaporating after applied. Other components such as dyes, resins etc. will start decomposing and polymerizing and the strokes becomes harder over time ⁽¹²⁰⁾. Nanotechnology and NPs plays an important role in the dating of the document and the examination of the age of ink. Various types of NPs are used in the ink formation, mainly in the colorants or the pigments of the ink, which helps in the identification and the age determination of the ink. The examination of ink is mainly based on chromatographic examination but the ink consisting the NPs can't be determined by chromatography. Hence to estimate the age of the ink containing NPs, SEM-EDX is the most reliable technique for the ink analysis ⁽¹²¹⁾. Besides the commonly used security features such as watermarks, fluorescent inks, security fibres, optical inks, holograms etc. various NPs such as TiO₂, CaCO₃ and BaSO₄, various QDs and fluorescent NPs are commonly used in the form of fillers and pigments in the papers. These can also use in the estimation of age of the document depending upon the time when they introduced ⁽¹²²⁾.

For the determination of ink strokes, Atomic Force Microscopy (AFM) works at nanoscale level to analyse the interconnection and the crossing over of inks by scanning the strokes. The AFM is designed to use the force of elements between the cantilever probe and the surface of the sample and then measure the shape of the surface ⁽¹²³⁾. The AFM can determine the chronological order of the printing of ink on the surface, without any damage. The AFM technique uses Diamond NP cantilever for the process of nano indentation in which pressure is applied and the hardness of the surface is measured, hence the area and the depth of the ink deposition is examined. The AFM produces 3D images of the examined surface. To identify any kind of forgery and alteration, scientists have developed Solvent free gold NPs assisted LDI-MS approach to detect the image of dyes and inks. SALDI-MS i.e., surface assisted laser desorption/ionization- mass spectroscopy can also be used with the help of metal, carbon, and silicon nanostructures for the Questioned document examination ⁽¹²⁴⁾. To detect the counterfeiting, various barcodes are used which are used to label, track, and authenticate goods or packages but these barcodes are facing increasing challenges of being falsified, altered and damaged. These barcodes face various limitations such as physical damage, the size of materials which are added to form the barcode etc. To overcome these challenges, nano barcodes are used as they have intelligent properties and applications ⁽¹¹¹⁾. In a recent study, Kim et al. describes about the NPs based barcode which operates via SERS ⁽¹¹⁵⁾. The Ag deposited Fe₂O₃ NPs were salinized to form Ag- Fe₂O₃ Raman markers. Ag- Fe₂O₃ barcodes can be formed to prevent counterfeiting and illicit activities. Another type of

barcode utilizes CoFe/Au nano barcodes which are fabricated using one bath electrodeposition ⁽¹¹⁶⁾.

Other modern technique such as surface plasmon resonance (SPR) methods and fluorometric methods which involves the use of fluorescent NPs and QDs for the detection of the counterfeit materials ⁽¹²⁵⁾. Various types of lanthanides doped luminescent NPs, semiconductors, CDs and QDs are used to produce various types of security inks which are used in the documents ⁽¹²⁶⁾.

- 7. Nano-Sensors and Nano-Trackers Used in Forensic Investigation:** Nano-trackers are the devices which are incorporated into the goods or items to prevent it from being stolen. These nano-trackers generate nano codes due to which it became possible to track the evidences. The nano-sensors use carbon, graphite, silica based QDs due to their unique optoelectronic property, less toxicity and availability for preparation ⁽¹²⁷⁾. The commonly used nano-sensor which is made up of novel Mn doped ZnS QDs, mainly helps in the detection of Cocaine and its derivatives from oral fluid and serum ⁽¹²⁸⁾. Detection of various poisons like Hydrogen sulfide can be done using carbon QDs sensors, Cyanide ions using graphene QDs sensors ⁽¹²⁹⁻¹³¹⁾, various volatile poisons such as methanol, ethanol, acetone, formaldehyde etc. can be detected using CdTe QDs sensors. Ammonia gas can also be detected using PbS QDs and TiO₂ nano-tubes ⁽¹³²⁾. Another method is the use of peptide based physical taggants which is physically marked into the object. These taggants will get transferred into the suspected object and upon analysis shows fluorescence. These taggants can also be recovered from the criminal or the person who is involved in the crime who comes in contact with the taggant article ⁽¹³²⁾. Nano-taggants are also used in the detection of explosive substances such as dynamite, PETN, etc. These taggants are isotropic taggants which is prepared for the unique identification of the isotopes of various explosives ⁽¹³³⁾. Nano-trackers now-a-days are kept inside the body of the prisoner, so that if he will try to escape or performs any kind of illegal activity after release from jail, then the policemen are able to keep an eye upon the criminals ⁽¹³⁴⁾.
- 8. Use of NPs in Fingerprint Identification:** Fingerprints (FPs) are the prints produced by the surface of the fingers on any surface which comes in contact with it. FPs are the most commonly found trace evidence in the crime scene which can become crucial evidence for the identification and individualization of the criminal. FPs are formed by the accumulation of salt and amino acids which are released via the sweat pores. Analysis of these FPs becomes a vital task in the crime scene investigation. FPs can be found on either porous or non-porous surface. Porous surface includes paper, cardboard, sponge etc. and non-porous surface includes wood, tiles, door, wall etc. FPs can be of two types i.e., Latent prints, which cannot be seen by naked eyes and patent prints which can be visualized by naked eyes ⁽¹³⁵⁾. Hence visualization of latent prints becomes a difficult task for the forensic examiners. There are various conventional methods for visualization of the FPs such as Iodine fuming method, Ninhydrin method, super glue fuming method, and various other physical developer methods. The latent prints can also be visualized by some physical methods such as applying various types of powders such as charcoal powder, metallic powder etc. The basis of these chemical reactions is the adherence of the powder and other chemicals upon the FPs but these materials have their own drawbacks. The major drawback is the visibility of the FPs which decreases when the time passes. Also, the size of the granules of powders are big enough that it cannot stick properly to

the FPs and the surface and the prints look the same as the background. Other risk is the contamination of the latent prints with the powder⁽¹³⁷⁾. Hence decipherment of the FPs becomes a difficult task. To overcome all these drawbacks, various types of NPs are used for the visualization of latent FPs. These NPs are very small in size, higher surface area, highly sensitive and give a great contrast with the latent prints. The small size of these NPs has a great advantage as it can bind with the minute ridges present in the FPs and can produce a clear, superior image of the ridge details along with the sweat pores as compared to the conventional methods used for visualization of the FPs⁽¹³⁸⁾. At the end of the year 1980s, Saunders used gold (Au) NPs for the visualization of latent FPs⁽¹³⁹⁾. The Au NPs are inert and the FPs developed by Au NPs can be stored for longer duration⁽¹⁴⁰⁾. The Au NPs have also an advantage over the conventional chemical methods. The chemical methods fail to visualize those types of FPs in which the salt and amino acid content are present in very less amount, hence Negative or Reversed Fingerprinting is done using the Au NPs. In this method, the reaction occurs between the ridges of the FPs (and not on the ridges of the FPs), which is done by using Au NPs followed by silver precipitation⁽¹⁴¹⁾. The Au NPs are suspended in petroleum ether, which adheres to the surface of the sweat residues through hydrophobic interaction. Upon development using silver precipitation, a dark impression of ridge details is produced which is clearly visible through naked eyes⁽¹⁴²⁾. Other NPs such as cadmium selenide/ zinc sulphide nanoparticles (CdSe/ZnS NPs) are also used for the detection of latent prints in non-porous surface. These NPs give fluorescence under UV radiation.

Another NPs such as Si NPs are also used for the development of latent FPs. These NPs are functionalized using organosilanes and conjugated by fluorescent dyes, which gives best result in development of latent prints⁽¹⁴³⁾. With the further advancement in the nanotechnology, QDs are also highlighted for their special optical properties for the development of latent FPs. Mainly used QDs are Cadmium sulphide, cadmium telluride and cadmium selenide. These cadmium based QDs are used over the conventional powder methods, but later, it is found out that these QDs are toxic in nature. Hence, in the replacement of this Cadmium based QDs, Zinc QDs are used and it is proven to be less toxic and more reliable⁽¹⁴⁴⁾. In a study, Wang et al. utilized magnetic NPs for latent FPs detection. The researchers used iron oxide magnetic NPs which are fabricated using co-precipitation method and then coated with silica. These NPs are then combined with CdTe QDs for better results and visualization.

The researchers took FPs on various surfaces and compared the visualization power of the conventional powders with the magnetic NPs. The combined magnetic NPs show good magnetic as well as fluorescence property due to the presence of iron oxide magnetic NPs. These NPs can visualize clear, partially damaged as well as damaged FPs with detailed features⁽¹⁴⁵⁾. In another research, Huang et al. uses a similar concept of combining the magnetic and fluorescent properties of NPs to detect latent prints. He combined platinum nanoclusters (negatively charged) with Fe₃O₄ magnetic NPs (positively charged) which is synthesized from Glutathione, which is a reducing and protective agent and Polyethyleneimine which is a modification agent. The combined particle is then named as Fe₃O₄@GSH-Pt Nanoclusters core shell microsphere powder. When this powder is brushed upon the latent print, a red coloured fluorescence is observed under the excitation of 465nm wavelength. The main principle behind this powder detection is based on two aspects, first is the electrostatic adsorption of Fe₃O₄

with grease, sweat and other compounds and second is the amino acid present in the FPs based on the empty orbital structure of GSH-Pt nanoclusters⁽¹⁴⁶⁾. This powder has more potential than other powders and can show good contrast, but the demerit of this powder is that this cannot be used in the case of old prints. Hence to overcome this challenge, Chen et al. synthesized surface-modified MNPs. He prepared Fe₃O₄ superparamagnetic NPs which are coated with polyethyleneimine. These PEI-Fe₃O₄ NPs gives better results and it overcomes the challenge as it can also used for aged FPs. Hence, PEI-Fe₃O₄ NPs is highly reliable and recommendable NPs which can be used in future for the purpose of FPs detection⁽¹⁴⁷⁾. Further, other methods are developed to detect the presence of old latent FPs. One of the methods include formation of magnetic NPs which are prepared by coating GSH-Au nanoclusters NPs over Fe₃O₄-PEI NPs to obtain a core-shell structure, which was called Fe₃O₄@GSH-Au Nanoclusters. This technique has the same principle as Fe₃O₄@GSH-Pt Nanoclusters but it is more reliable and can visualize latent prints up to 21 days on various surfaces⁽¹⁴⁸⁾. In the case of bloodied FPs, QDs are functionalized with up conversion NPs made from lanthanide doped rare earth metals such as NaYF₄: Yb, Er, up conversion nanomaterials. These NPs shows strong fluorescence and is less toxic⁽¹⁴⁹⁾. Song K et al. has explained a method for the detection of latent prints on the multicoloured surface. He used poly (styrene-alt-maleic anhydride)-b-polystyrene (PSMA-b-PS) functionalized gold NPs and done colorimetric imaging and photo acoustic imaging of the latent FPs⁽¹⁵⁰⁾. Besides the various types of NPs, CDs are also widely used in the development of latent FPs due to their highly fluorescence property. Fernandes et al in his research shows that when 0.7 wt. % of CDs is incorporated into silica matrix, then it shows highly detailed and colour tuneable ridge characteristics. These CDs are prepared by thermal treatment of citric acid monohydrate and ethanolamine, followed by dialysis against water⁽¹⁵¹⁾. An instrumental method is also developed for the visualization of latent FPs. This method is known as micro-X-ray fluorescence method. This method has several advantages and the major advantage is that this method is non-destructive. Another advantage is that adulterations and errors are reduced as spot detection is possible in this method⁽¹⁶⁰⁾. The below mentioned TABLE 4 shows the various applications and properties of NPs in the field of Forensic science.

Table 4: Application of NPs in Forensic Science⁽¹¹⁹⁾

S. No.	Branches of forensic science	Uses of NPs	Forensic significance	Properties
1.	Forensic biology and serology	Magnetic NPs such as iron oxide NPs, mesoporous silica-based NPs.	Separation of nucleic acids such as DNA and RNA.	Non-toxic, easy separation, time consuming process, requires no sample processing etc.
		α DNA-coated Magnetic NPs	To separate diatoms.	α DNA-coated Magnetic NPs shows high silica binding property in the presence of chaotropic agents.
		polyethyleneimine with phosphor tungstic-acid multilayer	To detect the presence of creatinine in urine.	Shows more accurate results than Jaffe's colour test.

		modified electrode		
		Au and Ag-NPs	Examination of blood	Unique optical properties.
		Electrochemical sensors made up of Pd, Pt and Au NPs. Au NPs are also used in combination with Carbon based NPs.	Examination of uric acid in blood serum and urine.	Large surface area, shows good conductivity and stability, have small dimensional size and excellent catalytic properties
2.	Forensic Chemistry	Nano-sensors, semiconductor CdS nano slabs, Au NPs, CDs etc.	Detection of explosive materials.	low cost, high \square sensitivity, low \square consumption of power, advanced stability, fluorescence properties etc.
		Fe ₂ O ₃ @ Poly (meth-acrylic acid-co-ethylene glycol Di methacrylate) resin, SiO ₂ -magnetic NPs based biosensors etc.	Detection of arson materials.	Fe ₂ O ₃ resins acts as sorbent.
3.	Forensic Ballistics	Au NPs based nano-sensors, carbon nano-tubes, CaO NPs etc.	Examination of Gun Shot Residue (GSR)	Ultra-sensitive, high surface \square area \square to \square volume ratio and quantum confinement.
4.	Forensic Toxicology	Iron oxide NPs, various magnetic NPs, functionalized NPs such as Graphene oxide, CNT electrodes, Au Nanoprobes etc.	Examination of illicit drugs.	Can detect drugs in trace levels.
		Magnetic NPs.	Examination of clinical medicines	Dispersibility, large surface area, surface tunability etc.
5.	Forensic Documents examination	Photonic crystals (PC), superparamagnetic magnetite (Fe ₃ O ₄) colloidal	Used for question documents analysis	PC allows the propagation of Electromagnetic waves having specific frequency.

		nanocrystal clusters (CNCs)		
		Copolymer latex nanoparticles chemically based on methyl methacrylate and butyl acrylate; Nitrogen doped CDs	Anti-counterfeiting Ink, invisible ink	Photochromism, excellent thermal and photostability performance, shows illumination in UV and visible region, has high quantum yield
	Counterfeiting detection	Ag deposited Fe ₂ O ₃ NPs, Co, Fe/Au nano barcodes.	To detect counterfeit goods and other products.	Shows high saturation magnetization.
7.	Fingerprinting	Au NPs, Si NPs, QDs, Fe ₃ O ₄ @GSH-Pt Nanoclusters core shell microsphere powder.	Detection of latent fingerprints.	Adherence and fluorescence properties, can detect very trace number of prints etc.

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