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Analytical Techniques for Biomedical Nanotechnology

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# Chapter 8

# Mass spectroscopy in biomedical nanotechnology

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In recent years, mass spectrometry (MS) has been extensively used in the characterization of various nanomaterials. MS is a very sensitive analytical technique with a number of applications in the various fields of science and technology. The high precision and resolution of the instrument have opened new opportunities in the examination of various biological compounds. In this chapter, first a brief introduction and historical background on MS is given, and then the fundamental and applied aspects of MS especially in the field of nanotechnology highlighting its versatile biomedical applications are covered. A specific consideration is given to the MS analysis of various nanoparticles such as silver, gold, metal oxides, engineered nanoparticles, tellurium biogenic nanoparticles, carbon-based nanoparticles, and their biomedical applications. The present status and the upcoming possibilities of the MS technique are examined in general along with appropriate examples drawn from the existing literature.

## Abbreviations

| | |
|---|---|
| MS | Mass spectroscopy |
| MALDI | Matrix-assisted laser desorption ionization |
| HDL | High-density lipoprotein |
| LDI | Laser desorption/ionization |
| LC–MS | Liquid chromatography–mass spectroscopy |
| MRI | Magnetic resonance imaging |
| SPR | Surface plasmon resonance |
| BBB | Blood–brain barrier |
| CNS | Central nervous system |
| MSI | Mass spectrometry imaging |
| HPCL | High-performance liquid chromatography |

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| | |
|---|---|
| GC/MS | Gas chromatography/mass spectrometry |
| SERS | Surface-enhanced Raman spectroscopy |
| CE | Capillary electrophoresis |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| RSD | Relative standard deviation |
| CHCA | α-cyano-4-hydroxycinnamic acid |
| MALDI-TOF MS | Matrix-assisted laser desorption/ionization-time of flight mass spectrometry |
| spICP-MS | Single-particle inductively coupled plasma mass spectrometry |
| ICPMS | Inductively coupled plasma mass spectrometry |
| FFF | Field-flow fractionation |
| HDC | Hydrodynamic chromatography |

## 8.1 Introduction

Mass spectroscopy (MS) is a highly sensitive technique providing fast data acquisition speed and analysis of samples up to the picogram level [1–3]. It is one of the most significant conventional spectroscopic analytical tools and has been generally employed to investigate the chemical structures of organic materials and small biological molecules [3]. In addition, MS can give information on the molecular mass of a sample. MS has also been broadly used to dissect biological molecules and has developed into an irreplaceable tool for proteomics research [4]. The technique is being widely used in genomics and proteomics because of its high rate of reproducibility, reliability, broad application, and benefaction in modern biomedical sciences. The basic principle of MS lies in the effective ionization of chemical compounds followed by the separation of ions on the basis of mass to charge ratio [5]. Molecules are fragmented by the generation of ions by employing an energy and the formed ions are then isolated in the analyzers in the presence of electric and magnetic fields and subsequently recorded by the detector to give rise to a mass spectrum on the basis of their mass/charge ratio (figure 8.1) [2].

In this way, every molecule of the compound either purified or homogenate can easily be recognized and further its quantification can be done. To explain it in a simple way, a mass spectrum quantifies the mass in a sample via charging them during the measurement. To date MS has been utilized in numerous fields such as applied sciences and basic sciences including biomedical sciences. The identification of disordered proteins or metabolites for quantitative and qualitative analysis is possible using mass spectroscopy under experimental conditions [5].

The history of MS dates back to the 18th century by Eugen Goldstein. The mass spectrograph was developed by J J Thompson thereby providing a major contribution to the development of MS for various analytical applications [2, 3]. The word spectrograph derived through these devices was introduced internationally to the scientific community and was used in the scientific vocabulary from 1884 [6–8]. The modern MS techniques offer a more accurate analysis of atomic masses and structures of various compounds. Moreover, the discovery of new soft ionization techniques like electrospray mass ionization and matrix-assisted laser desorption

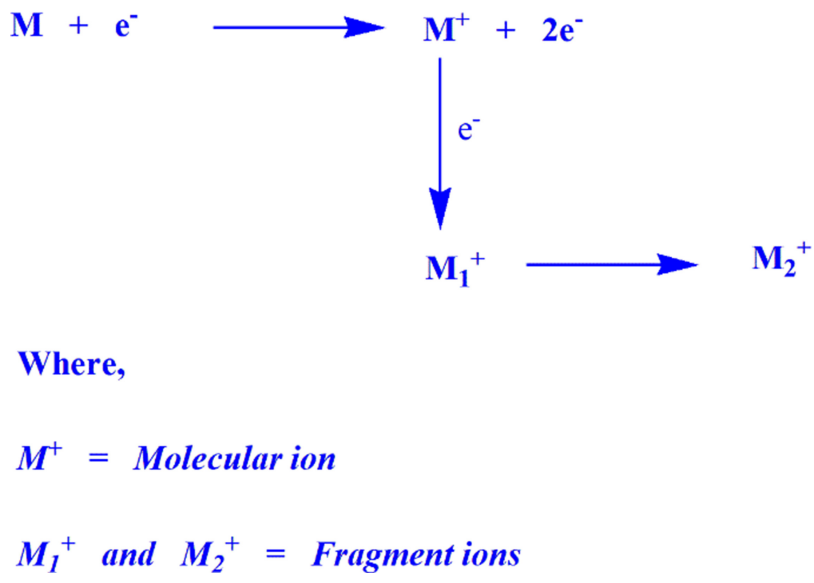


**Figure 8.1.** Ionization of molecules by energy application (here electron bombardment). Reproduced from [2]. CC BY 3.0.

ionization (MALDI), have made MS more precise in the analysis of the mass of high molecular weight compounds like polymers, nucleic acids (DNA and RNA), etc [3, 9–11]. MS has many advantages and has the potential to replace many techniques in the future. In the field of pharmacokinetics, MS is found to have a unique importance because of the complexity that the matrix provides for the analysis of samples like blood and urine [5]. Tandem MS is also utilized by researchers these days as it adds specificity to the drugs [12–14]. The pharmacokinetics of drugs present at various time intervals as well as their rate of clearance from the body can be analyzed and regulated via blood and urine [2]. However, the analyzers present in MS are highly sensitive which in turn efficiently helps the pharmacologists to study micro-dosing procedures. The wide implementation of MS will continue to benefit the advancement of a deep understanding of different biological processes followed by their molecular mechanisms. Additionally, MS-based proteomics and metabolomics have helped provide a deep understanding of various biologically occurring processes along with their mechanisms which will be helpful in clinical molecular diagnosis in the near future. Even in medical sciences, MS is useful for the examination of metabolites and the determination of the presence of abnormal proteins in patients [15, 16]. It requires a small sample for analysis and provides high sensitivity and precision to the analysis. All of the reasons stated above make MS the most reliable technique to be used in various laboratories worldwide [17–19]. MS has been used in the analysis of respiratory gas in patients and has shown accurate results [20]. It has been found that some of the MS instruments have been modified intentionally for reporting the composites of gases which are respired by a patient within a mass range of detection [21]. The highly accurate results obtained from MS have made

it useful in the quantitative analysis, characterization, and sequencing of proteomic studies. With the help of electrospray ionization and MALDI, it is possible to fragment the proteins which can further be digested using trypsin enzyme followed by analysis for mass fingerprinting [8, 22]. Zhang *et al* [23] reviewed the studies on metabolomics for biomarker discovery demonstrating its role in the identification of biomarkers. Cheng *et al* [24] provided a detailed review of the developments of mass spectroscopy-based qualitative proteomics techniques, high-density lipoprotein (HDL) proteomics, and modification of lipoprotein for the discovery of a biomarker for the treatment of vascular disease—atherosclerotic. Godderis *et al* [25] demonstrated the mass spectroscopic application over the evaluation of DNA methylation and hydroxymethylation. They utilized the liquid chromatography–mass spectroscopy (LC–MS) method for quantification as well as for the simultaneous comparison of global methylation and hydroxymethylation in human DNA present in various tissues. In glycobiology, MS is found to be more reliable for the examination of the properties and structure of glycans [26]. The measurement of masses using laser desorption/ionization (LDI)/MALDI MS and tandem MS is considered to be reliable for the identification of structures of peptides, carbohydrates, and lipids as they play an important role in biomedical applications having a molecular mass greater than 1000 Daltons. MS is now gaining a lot of attention for the characterization of nanoparticles (NPs). It provides information about the mass of ligands [27]. The combinations of LDI MS with MALDI has been successfully used for the determination of masses of small ligands present in gold (Au) NPs in biological samples [28]. The basic principle behind the working of the LDI/MALDI method is that the NPs tend to absorb energetic laser irradiation thereby causing ligands to ionize and be released to be detected by MS [28]. Nanotechnology has many applications in the research and development of matter at the macromolecular or atomic level having dimensions in the range of 1–100 nm. The materials at this scale show different quantum mechanical properties. The so-formed NPs need to be characterized for their functionality and toxicity. Therefore, the use of MS for the complete in-depth analysis of the structures with nanometer dimensions as well as the characterization of the surface composition of NPs is found to be highly beneficial in this area. This chapter deals with the detailed use of MS in the analysis of NPs for various biomedical applications. A detailed overview of all types of NPs and their characterization using MS has been discussed here.

## 8.2 Biomedical applications of nanoparticles

Nanoparticles can be defined as solid colloidal particles having a diameter range between 1 and 100 nm. Their wide employment for biomedical applications is due to the fact that they provide various advantages to the large-sized particles like large surface area and increased magnetic properties [29]. Recently, NPs have attracted a lot of interest in numerous biomedical applications like targeted drug delivery, photoablation therapy, hyperthermia, biosensors, and bio-imaging [30, 31]. NPs can be classified into four types of nano-systems, i.e., bimetallic nanoparticles, metallic nanoparticles, magnetic nanoparticles, and metal oxide nanoparticles as shown in figure 8.2, with each category contributing towards the applications in biomedical science.

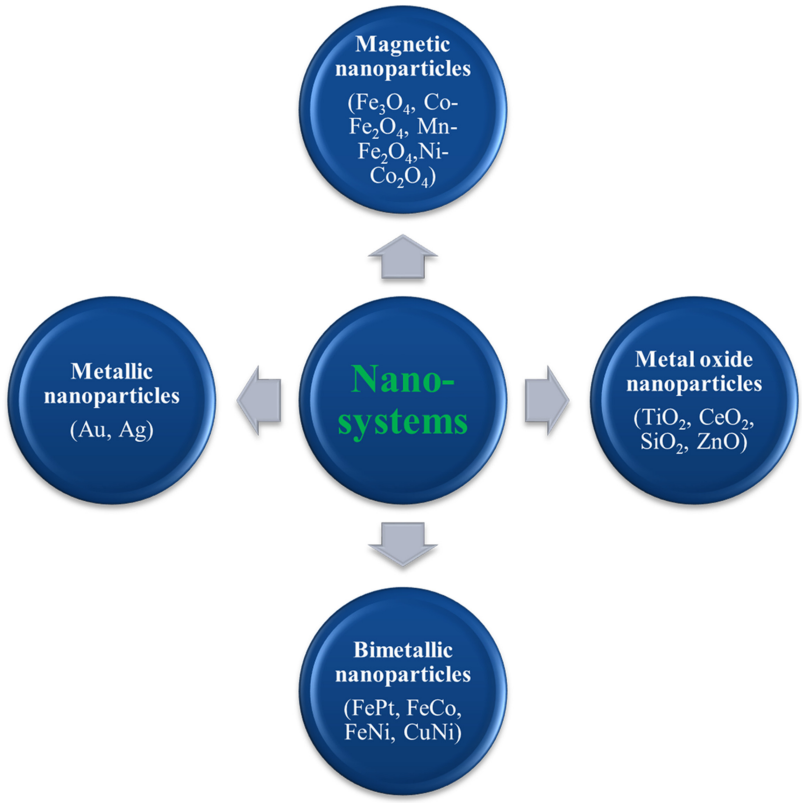


**Figure 8.2.** Schematic representation of classification of nano-systems.

### 8.2.1 Magnetic nanoparticles

These are considered to be the most common type of NPs employed for biomedical applications because of their distinctive chemical structure, biocompatibility, non-toxicity, chemical stability, high saturation magnetization, and high magnetic susceptibility [32]. Iron oxide comprises multiple states of oxidation like iron (II) oxide (FeO), iron (III) oxide ($Fe_2O_3$), and iron (II, III) oxide ($Fe_3O_4$). $Fe_2O_3$ consists of separate crystalline polymorphs of α-$Fe_2O_3$, β-$Fe_2O_3$, γ-$Fe_2O_3$ and ε-$Fe_2O_3$. Maghemite (γ-$Fe_2O_3$) and magnetite ($Fe_3O_4$) is found to be highly biocompatible, however, $Fe_3O_4$ has been used widely in biomedical applications [33]. It is easily oxidized; to prevent this coating on its surface with biocompatible shell-like metals, polymers, and ceramics are required [34–36]. Once the surface is shell coated, it provides many advantages like prevention of agglomeration, functionalization, and conjugation to enzymes, antibodies, anticancer drugs, and proteins. FeO NPs are effectively used for treating hyperthermia [37, 38], drug delivery [39–45], and magnetic resonance imaging (MRI) [46, 47]. Furthermore, the magnetic properties of FeO NPs can be enhanced through mixing with susceptible elements like cobalt, nickel, and manganese [48]. Of these, cobalt and manganese are widely used in the

field of biomedical sciences. The $CoFe_2O_4$ nanoparticles consist of very large magneto-crystalline anisotropy, high Curie temperature, coercivity, considerable chemical stability, and moderate magnetization saturation [49–53]. The major applications of these NPs lie in the treatment of hyperthermia as well as important contrast agents for MRI [49–53]. Similarly, $MnFe_2O_4$ NPs show high magnetization, biocompatibility, and magnetic susceptibility. These have also been used for the possible treatment of magnetic hyperthermia [54–57].

### 8.2.2 Bimetallic nanoparticles

These include iron and cobalt-based NPs which show excellent magnetic properties like high Curie temperature, superparamagnetism, and high saturation magnetism. Despite these advantages, they have the major limitation of being easily oxidizable putting their biocompatibility into question. Coating the surface of NPs with some biocompatible material is the solution to the above problem. The iron–cobalt NPs have been utilized for many applications like contrast agents for MRI owing to their property of high saturation magnetism and hyperthermia [58–64]. Iron nickel-based NPs consist of a high Curie temperature along with some magnetization properties used majorly for MRI [65]. Copper–nickel NPs also come under bimetallic NPs having good magnetic properties being used for biomedical applications [65–68]. Bimetallic NPs like iron platinum (Fe–Pt) show a variety of chemical and magnetic properties like high Curie temperature, chemical stability, saturation magnetization, superparamagnetization, and high x-ray absorption [69–72]. It is due to these properties that the bimetallic NPs are ideally used for the treatment of hyperthermia [73, 74], MRI contrast agents [75–78], drug delivery [79–83] and biosensors [84].

### 8.2.3 Metallic nanoparticles

A new field of exploration has grown recently called ‘plasmonics’ that manages surface plasmon resonance (SPR) in the case of noble metals such as gold (Au) and silver (Ag) structures at the nanoscale dimensions. They show remarkable properties with a decrease in size and they have a large specific surface area, a high fraction of surface atoms, and large surface energy. These interesting properties of NPs have motivated researchers to use them in a variety of applications. Au and Ag NPs are being used for medical purposes. They have unique optical properties, electronic properties, chemical inertness, and an ability for surface functionalization because of the presence of negative charge over their surface [85–87]. They have been widely used for bio-imaging [88, 89], biosensors [90, 91], and photothermal therapy [92–94]. Au-NPs tend to conjugate easily with antibodies, drug molecules, and ligands for active or passive delivery [90–99]. The property of chemical inertness makes Au-NPs highly compatible with *in vitro* and *in vivo* applications. Similarly, Ag NPs have also been studied for various medicinal purposes because of their physicochemical properties like high electrical conductivity, chemical stability, thermal conductivity, chemical stability, antibacterial activity, catalytic activity, and optical properties [100]. The main applications of Ag NPs lie in electronic, antimicrobial, photonic, and disinfectant fields [101–108]. Ag NPs hold the excellent capability of

antimicrobial activity which is why they are primarily used in wound dressings, textiles, and device coatings. Apart from this, they are also being used as biosensors [101–103], photothermal sensors, and targeted drug delivery applications [109–111].

### 8.2.4 Metal oxide nanoparticles

Titanium dioxide ($TiO_2$), cerium oxide ($CeO_2$), silicon dioxide ($SiO_2$), and zinc oxide (ZnO) are a few of the metal oxide-based nanoparticles used for a number of biomedical applications. Starting with $TiO_2$ NPs, they have important uses in various photovoltaic and photocatalytic devices, paint, food coloring, cosmetics, and toothpaste. They possess various properties like biocompatibility, optical properties, and chemical stability [112]. Due to these excellent properties, $TiO_2$ has been used for biomedical applications such as bio-imaging [113], drug delivery [112–117], biosensors [118], and photoablation therapy [119]. $CeO_2$ NPs are also known as nanoceria and have a characteristic switching properties in various oxidation states [120]. They report certain defects on their surface including oxygen vacancies resulting in a state of mixed valency of cerium (IV) and cerium (III) oxidation states coexisting on the surface. This leads to the nanoceria gaining catalytic activity. This has resulted in nanoceria being a prospective biological antioxidant. Nanoceria has been used as a biosensor and as an anticancer agent in various applications [121]. Another class of metal oxide NP includes $SiO_2$ NPs. These consist of large specific surface area, pore-volume, adjustable particle size, and great biocompatibility. Therefore, these properties of mesoporous silica make them an ideal material for drug delivery and bio-sensing applications [122, 123]. The use of ZnO NPs has become important recently but the surface of ZnO NPs is protected by a modification to prevent them from dissolving into acidic and neutral solutions. In order to use these NPs in fluorescence imaging, doping is required because they comprise of bandwidth in the UV region which makes it impossible for the UV light to penetrate the tissue [124].

## 8.3 Mass spectroscopy in biomedical applications

### 8.3.1 Mass spectroscopy for the detection of nanoparticles in neuroscience

The human brain is regarded as the controlling unit for the entire body, regulating the functioning of the organs and the entire system. With changes in lifestyle and an increase in life expectancy, the brain has been subjected to a prolonged aging process leading to a high risk of developing neurogenerative disorders like Alzheimer's, Parkinson's and dementia. Despite advancements in medical science, no effective therapy for the treatment of these neurogenerative disorders has been discovered because of the restriction imposed by the blood–brain barrier (BBB) [125–129]. The BBB is a semi-permeable membrane formed through tight junctions present on the endothelial membrane helping in the separation of blood vessels and the central nervous system (CNS) [125–129]. The major function of the BBB is in the protection of blood and blood vessels from the passage of harmful pathogens, toxins, or hormones. The BBB helps protect the brain by limiting the passage of toxins, hormones, and pathogens present in the blood. In a similar way, this filter inhibits

the passage of therapeutic agents in reaching the brain, thereby prohibiting their treatment. The transmission of the drug across the BBB is crucial for the treatment of diseases like cancer, Parkinson's, Alzheimer's, and other neurological disorders. Recently, NPs have gained importance in the active release of drugs at targeted sites because of their ability to maintain drug levels in the therapeutic range and increasing their half-life, permeability, stability, and solubility [130]. NPs can be synthesized from various materials including polymers, lipids, proteins, plants, flowers, and semiconductors, thereby making their physicochemical properties suitable for targeted drug delivery as well as for imaging and therapeutic uses [131]. Therefore, monitoring of the working efficiency of NPs as drug carriers is an important step in the treatment of diseases and clinical translation. The need for an appropriate method which helps in imaging the nanocarriers in a certain interval of time will be helpful in keeping a check on the efficacy of the drug and treating the disorder.

Mass spectrometry imaging (MSI) has great importance in medical and clinical research with the detection and analysis of nanoparticles [132–134]. It has been investigated as an interdisciplinary technique consisting of physics, chemistry, and biology for analysing the molecular composition of various compounds [132–135]. MSI, a new technique that has been developed from MS, is useful for the visualization and study of the spatial distribution of various biological substances including proteins, peptides, metabolites, drugs, and lipids present in biological specimens. The molecular mass of a sample is taken as an intrinsic molecular parameter for label-free imaging using MS for the visualization of the distribution of biomolecules on the sample surfaces. The electric and magnetic fields are manipulated for this purpose and then the measurement of time of flight is done. The utilization of a mass spectrometer as an imaging instrument is done by equipping it with a desorption source and an ionization source which generates local information, an automated sample or beam manipulation system, automatic data acquisition, registration system, and visualization software. The signals so obtained are directly proportional to the compound concentration persistent in any sample. The mass spectra of each molecule represent its composition at $x$ and $y$ coordinates. An image dataset similar to pixels is obtained from the mass spectra of the tissue sample in the form of a digital photograph. Selective distribution of the compound inside the tissue section is visualized according to the mass to charge ratio at specific intensities noted from the obtained spectra. The amount of ion present in each pixel is visualized with the help of a color intensity scale in 2D maps. The data is extracted from the mass to charge ranges from the spatially obtained mass spectroscopy files to generate an image. Mass spectroscopy imaging is a suitable technique for studying the biodistribution in both large whole-body tissue samples as well as in small organs such as the liver, kidney, and eyes with a relatively high resolution [132].

Although the BBB restricts the entry of several molecules through them the employment of numerous methods has allowed the transit of drugs. Molecules smaller than 500 Dalton, being highly soluble in lipids, can efficiently penetrate the BBB barrier through passive diffusion. Using this strategy drugs can be easily delivered to the targeted site using liposome-based particles [136]. NPs can be easily fabricated up to nanoscale ranges and these are considered to be the most dominant

ways to pass through the BBB and successfully reach the target site. All the physicochemical properties of NPs can be exploited for the facilitation of drug release at the target site with an optimal value. Till now the fluorescence imaging technique has been used for the detection of NPs inside the cellular organelles in cells. However, this technique has the disadvantage of not being able to carry out the imaging of the carrier and cargo molecules concurrently within the cell [137, 138]. To circumvent the disadvantages posed by the fluorescence imaging techniques, MSI is used. A three-dimensional molecular image of the cells and analytes of interest are easily obtained using MSI. The visualization of co-localized images of NPs along with the drugs of interest at a submicron level with a resolution at nanometer depth is possible using MSI. Figure 8.3 demonstrates an example of imaging of a composite Nano SIMS image of the HeLa cell lines incubated with 15 μM Cy15N-NP where 195Pt (red) and 15N enrichment (green) are overlaid on the image of secondary ion [138]. Yellow regions present specify the co-localization of

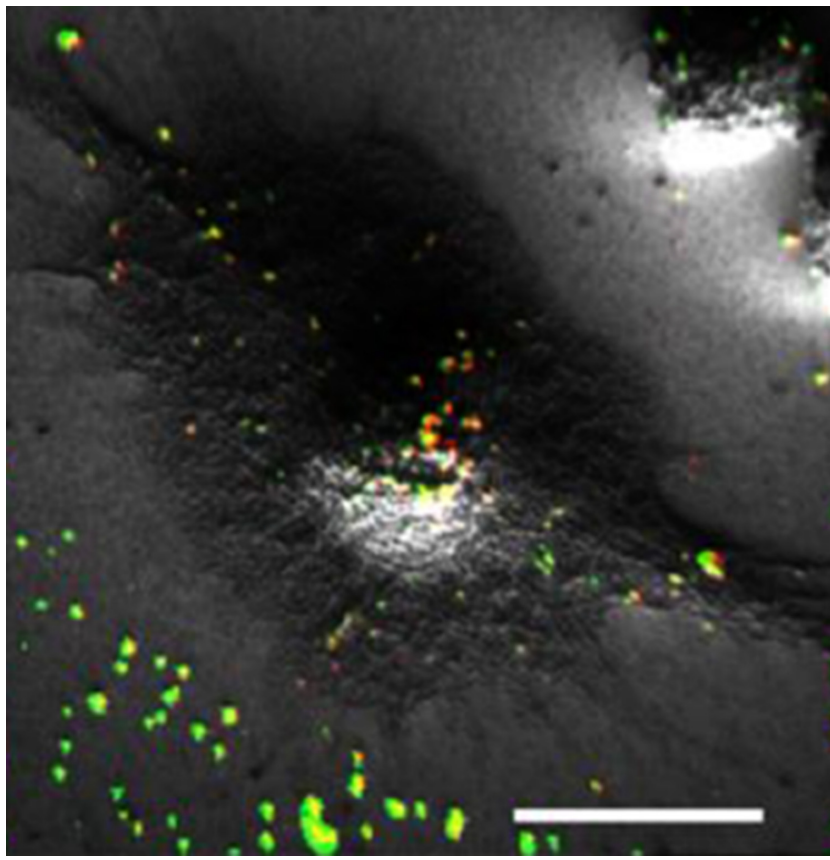

**Figure 8.3.** Composite nano image of a HeLa cell incubated with 15 μM Cy$^{15}$N-NP where $^{195}$Pt (red) and $^{15}$N enrichment (green) and overlaid on the secondary ion image. Yellow regions indicate co-localization of signals. The scale bar represents 10 μm. Reprinted with permission from [138]. Copyright (2016) American Chemical Society.

signals. The scale bar represents 10 μm. Therefore, the use of isotope labels is not required as the drug and its metabolites can be simultaneously detected using MSI. It can also be helpful in encountering problems with overlapping cell peaks. Apart from these qualitative characteristics, a quantitative estimation of the drug levels in tissue can also be done using MSI. Despite the wide variety of techniques used previously such as autoradiography, bioluminescent, and fluorescent receptors for the quantification of drugs in cells, they have many shortcomings which is why an alternative technique with high efficacy is required, so MSI offers various advantages in this regard. MSI has been reported to show the mechanism of optimization of designing therapeutic NPs along with the monitoring of pathogenesis of several neurodegenerative diseases thereby, making major contributions in neurosciences [138].

Xue *et al* [139] developed a novel label-free laser desorption/ionization MSI method for the imaging and measurement of the *in situ* drug release in tissues via examination of the intrinsic MS signal intensity ratio of the loaded drug over the nanocarriers. They loaded the anticancer drug doxorubicin (DOX) in $MoS_2$ nanosheets. They employed a label-free method for the simultaneous imaging of nanocarriers and release of drug as well as quantifying the amount of drug release in the targeted tissues. Furthermore, $MoS_2$-based nanocarriers additionally displayed extraordinary potential in gene therapy and other joined treatments [139–141]. Furthermore, large numbers of inorganic nanocarriers, such as Au-NPs, carbon nanotubes (CNTs), and phosphorus nanosheets, have been created and applied to biological frameworks, as a result of their high drug-loading ability and particular points of interest in combined therapy [142, 143]. Therefore, the trialing of these nanocarriers and loaded drugs in living systems is vital for biomedical investigations [139].

### 8.3.2 Mass spectroscopy for the analysis of sulfur drugs and biothiols using silver nanoparticles

It is a well-known fact that sulfur-containing peptides, amino acids, and drugs play an important role in human biological systems [144, 145]. For example, the homocysteine level present in blood serum is considered to be a risk factor for cardiovascular and Alzheimer's diseases. Furthermore, cysteine deficiency causes numerous health problems [146, 147]. A rise in the levels of thiol-containing amino acids and peptides in plasma are thought to cause HIV AIDS [148]. It is because of their crucial roles in the biological system a lot of emphasis is given to the detection of peptides and thiol-containing amino acids. Various analytical approaches have been employed for the detection of these compounds like high-performance liquid chromatography (HPLC), gas chromatography/mass spectrometry (GC/MS), surface-enhanced Raman spectroscopy (SERS), capillary electrophoresis (CE), and fluorescence measurements. However, these tools offer various disadvantages including extra derivatization steps before the analysis, lesser sensitivity and selectivity for the identification of biothiols from biological samples. MALDI MS is considered to be a highly reliable technique for the speedy analysis of nucleic acids, proteins, drugs, peptides, and environmental samples [149, 150]. The use of NPs as matrix and affinity probes for the analysis of peptides and proteins in

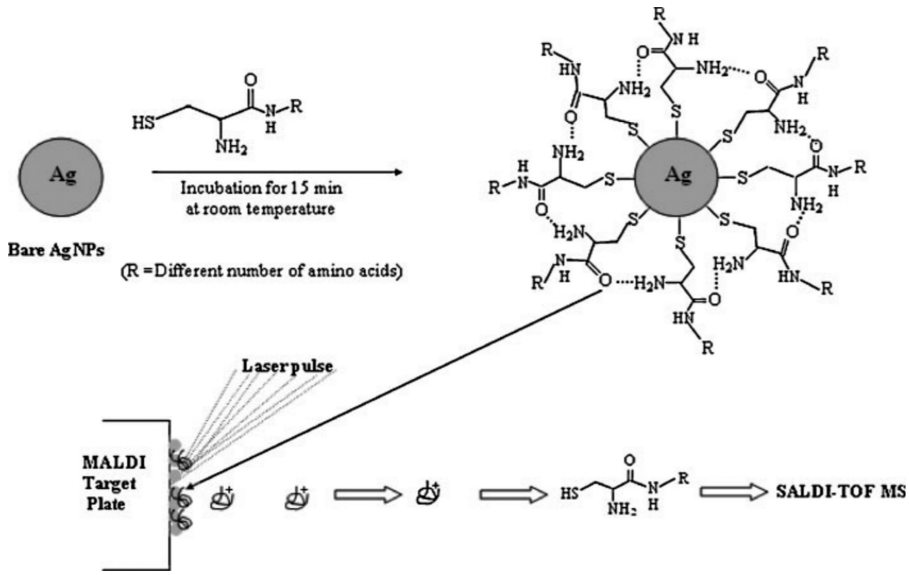


**Figure 8.4.** Diagrammatic representation of silver nanoparticles as preconcentrating probes and matrix for the SALDI-TOF mass spectroscopic analysis© of biothiols. Reprinted with permission from reference [151]. Copyright © 2008 John Wiley & Sons, Ltd.

MALDI MS is now becoming a popular technique. Kamlesh *et al* [151] worked on the synthesis of Ag NPS as matrix and affinity probes in MALDI MS for the determination of biothiols. The main reason behind using Ag NPs was that they have more affinity towards the sulfur atom and the –SH groups of cysteine-containing proteins and peptides can very well get attached to its surface via covalent bonding [152, 153]. They also proved the feasibility of the research by examining the urine samples collected from five volunteers. The interaction of Ag NPs with biothiols and their analysis using MS has been demonstrated in figure 8.4. The optical properties of Ag NPs along with the number of molecules adsorbed directly demonstrate the efficiency of ionization of biothiols. The values of the limit of detection (LOD) and limit of quantification (LOQ) for both the samples in urine, i.e., cysteine and homocysteine were calculated to be 7 and 22 nM, reporting a relative standard deviation (RSD) less than 10%. Upon the adsorption of biothiols over the surface of silver nanoparticles, an enhancement in the sensitivity of biothiols was reported to be around 4–15 fold. MS method offers various advantages over conventional methods such as simplicity, sensitivity, and rapidity for the examination of biological samples as it provides a high surface area and great photochemical properties for the Ag NPs which in turn effectively cause the release of drugs at the targeted sites.

### 8.3.3 Metal oxide nanoparticle-assisted laser desorption/ionization mass spectrometry for various medical applications

NPs having a core of metal oxides can successfully assist as analytes for ionizing molecules in LDI MS in the presence of glycerol [154]. For the examination of low

molecular weight samples, ZnO NPs are found to be convenient using LDI MS [155]. The limitation of using ZnO NPs was that their shape was anisotropic (a combination of cubic and rectangular) and they have a large variation in size, i.e., from 20 to 200 nm. The utilization of uniform nanoparticles in MS analysis is yet to be explored. Shu *et al* [156] prepared MnO NPs which have a core of metal oxide coated with a functional silicate sheet. The purpose behind coating the NPs with a functional sheet is that it improves the transfer of energy between the metal oxide core and analyte meaning the analyte is only charged/ionized via nanoparticles. The synthesized NPs were dispersed instead of aggregated. The results obtained from UV absorption studies at 337 nm proved that the MnO NPs can be used as an ionization supporting reagent of analyte for the nanoparticle-assisted LDI MS. The value of zeta potential was calculated to be more than +30 mV which was regarded as the threshold value for electrostatic stabilization [157]. The efficiency of the NPs to be used as an ionization material was investigated via a reserpine drug, which is a substance P acetate hydrate peptide and insulin protein [158]. They found that the oxidation of reserpine happened because of air and light. Although it did not yield any strong signal. When α-cyano-4-hydroxycinnamic acid (CHCA) was added, a few signals at a low molecular range were observed. The results obtained proved that NPs can be used as ionization-assisting reagents for a variety of analytes. Therefore, it was proved that magnesium nanoparticles will be more satisfactory for analyzing the low molecular weight samples [156].

### 8.3.4 Mass spectrometry imaging of *Lepidium meyenii* using gold nanoparticles

*Lepidium meyenii* Walp also known as maca is a functional food having medicinal properties and has a high economic value. It has been consumed in Peru for 2000 years as a source of food and medicine. Maca contains various secondary metabolites in its hypocotyls region like glucosinolate, flavonoid, steroid, and alkaloid. Alkaloids have many effects on the health of humans and animals due to the presence of natural organic nitrogen bases. It also has aphrodisiac properties because of the presence of fatty acids and macamides [159–167]. Even the pentane extract of maca is comprised of macamides which in turn act on the endocannabinoid system via inhibitory activity on fatty acid amide hydrolase [168]. Many activities of the CNS are exerted by maca extract. Macapyrrolin present in maca having a benzyl substituent attached to the nucleus of pyrrole has been studied for its cytotoxicity against five human cancer cell lines [165]. The above-stated properties of maca make it an excellent material for the treatment of diabetes, female menopausal syndrome, benign prostatic hyperplasia, and osteoporosis in the elderly. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) is used to analyze and locate the components of various molecules efficiently. Sihou *et al* [169] utilized Au-NPs as MALDI matrix for the analysis and location of components in maca root by MS. The size of the Au-NPs was calculated to be around 7–10 nm and because of the high absorption coefficient, they were utilized for MALDI mass spectroscopic examination of small molecules. The MALDI-TOF mass spectroscopy was used to distinguish between fresh maca

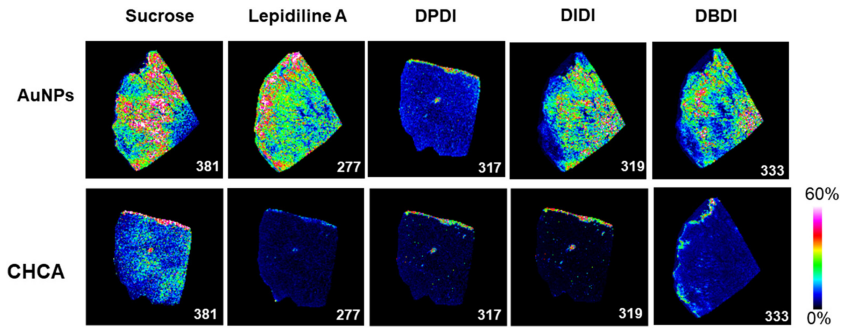


**Figure 8.5.** The ion distribution of compounds in maca root with gold nanoparticles and CHCA as matrices. Reprinted from [169], Copyright (2019), with permission from Elsevier.

and dry maca. The composition of maca having various constituents like sucrose, arginine, aspartic acid, etc using Fourier-transform ion cyclotron resonance mass spectroscopy. The use of Au-nanoparticles along with CHCA were used as matrices for the analysis of sucrose and alkaloids in dry maca root and the images proved that gold nanoparticles provided clearer images and spatial distribution in the efficient analysis of sucrose as demonstrated in figure 8.5. The position of various compounds in maca root tissue is vividly observable *in situ* by MALDI-MSI with gold nanoparticles as a matrix. Therefore, the use of MSI again provided a novel way of analyzing the medicinal properties of a compound for its use in the future for various applications [169].

### 8.3.5 Mass spectroscopy for the characterization of engineered nanoparticles

Engineered nanoparticles are being used in numerous consumer products which are currently on the market [170–173]. The most frequently employed inorganic nanomaterials include metallic NPs like Au and Ag, metal oxides such as $TiO_2$, and ZnO as discussed in section 8.2. The release of NPs from embedded matrices or as free entities is an area that needs to be analyzed using appropriate and sensitive instrumental techniques. The conventional techniques are not used for the characterization of NPs because of their unique physical and chemical properties. A well-established technique called single-particle inductively coupled plasma mass spectrometry (spICP-MS) is considered to be useful for the analysis of engineered NPs [174–177]. Its reliability, speed, and ability to size particles present at their lowest concentrations along with the side by side distinction between dissolved and particulate analytes make it an effective technique. If a separate separation step is required, the supplementary information about the nanoparticles can be obtained using spICP-MS. Therefore, obtaining information about size and quantifying the nanoparticles can be properly done if ICPMS is conjugated to some continuous separation techniques like field-flow fractionation (FFF) or hydrodynamic chromatography (HDC). When it comes to the synthesis of engineered NPs, size plays an important role [178, 179]. The shape of engineered NPs also plays a crucial role in a variety of applications like drug delivery, transport, targeting, and internalization

[180, 181]. Additionally, the chemical composition is also a crucial factor that is responsible for determining the toxicity of NPs [182]. Other properties like surface energy, wettability, surface charge, species absorbance, or adhesion are also considered to be some important parameters [183, 184]. The physicochemical characterization of the nanomaterials must be done by different physical states of engineered NPs, i.e., whether present in the dry powder form, solution form, or in suspension form and for this matter techniques for the determination of shelf life of engineered nanoparticles is essential [185–187]. The inorganic engineered NPs are characterized using the ICPMS technique as it provides very low detection limits up to nanograms per liter level. It usually involves the use of liquid phases which is why the solid samples need to be converted into a liquid through any process to be analyzed using the ICPMS technique. The practicability of direct study of suspension by ICPMS depends on the size and composition of the NPs [188]. It has the capability of carrying out rapid multi-element determination at the ultra-trace level making it beneficial for a variety of applications in various fields including environmental, chemical, medicinal, nuclear, metallurgical, biotechnology, and forensic science for toxicological and heavy metal poisoning studies. This technique is considered to be an innovative and emerging analytical approach for providing information about the elemental chemical composition of nanomaterials as well as their number concentration, size, and number size distribution. This is done on the basis of differences in signal to noise ratio produced for each particle. The spICP-MS technique has proven to be a highly reliable form of MS for the analysis of engineered NPs being further use for various applications. Mitrano *et al* [189] identified and quantified engineered Ag NPs in influent and effluent samples from wastewater treatment plants as the usage of consumer products can cause diseases in humans after their release into the environment. spICP-MS is capable of rapidly providing information about the size, distribution, and particle number concentration, elemental composition requiring minimal sample preparation providing high throughput and elemental specificity. It requires no instrumental modification and high detection ranges in terms of nanometer ranges [190].

### 8.3.6 Mass spectroscopy for the elucidation of tellurium biogenic nanoparticles

Tellurium usually designated as Te is a metalloid that belongs to group 16 in the periodic table and is used widely as an additive for manufacturing industrial materials like steel, optical disks, and solar panels [191, 192]. The metabolism of tellurium into organotellurium compounds is possible in various plants and one such widely studied plant for the metabolism of Te is garlic (Allium sativum) which is known for metabolizing various chalcogens like sulfur and selenium [193]. Tanaka *et al* [194] studied the elemental analysis of Te to determine its concentration in various parts of garlic using the ICPMS technique. The Te NPs were formed through the biological redox reactions in order to reduce its toxicity [193]. It is found that unambiguous observations of biogenic nanoparticles of tellurium were still lacking and there was a need for the investigation of the formation of insoluble NPs in garlic. The NPs were extracted from the clove and root samples by enzymatic

digestion and were analyzed using ICPMS. Additionally, the tellurium oxyanions get converted into a zerovalent form in garlic whose reaction mechanism was still unexplained [194].

### 8.3.7 Mass spectrometry analysis of carbon nanomaterials for various applications

Carbon-based nanomaterials such as CNTs, carbon nanodots, and graphene have been widely used for a variety of applications in the field of tissue engineering, drug delivery, bio-sensing, and diagnostics [133, 195–208]. Currently used techniques for imaging of carbon nanoparticles include Raman spectroscopy, near-infrared fluorescence, and transient absorption, however, these techniques have certain limitations like weak signals, strong background interference, and slow imaging speed making them undesirable for the studies [201, 202]. LDI MS is currently being used for the analysis of a variety of biological components [133, 203]. Xu *et al* [209] worked on the employment of CNTs as an effective matrix in matrix-assisted laser desorption ionization mass spectroscopy to analyse small biomolecules. The CNTs acted as sample carrying matrices upon which the sample solution was dropped. This simplifies the sample preparation steps, helps in the reduction of background interference, and improves sensitivity [1]. Dong *et al* [210] prepared a graphene-based matrix for the characterization of small molecular compounds like steroids, polyamines, anticancer drugs, and nucleosides by MS. Hua *et al* [211] used graphene nanoflakes as a matrix for the determination of cancer cells using mass spectroscopic analysis. These produced a very good quality mass spectrum having no background noise thereby making the study successful. Chen *et al*, [212], employed carbon nanodots to evaluate low molecular weight molecules via MALDI-TOF mass spectroscopy using both positive and negative ion modes. They carried out successful quantification of glucose and uric acid present in a biological fluid. The matrix was found to be highly efficient in the analysis of a variety of molecules consisting of peptides, amino acids, β-agonists, and oligosaccharides. Polystyrene-based carbon nanotubes were utilized as a matrix material for LDI MS which studied the quantification of urine and water samples utilizing benzo[a]pyrene (BaP) and 1-hydroxypyrene (1-OHP) as probes and acted as a sorbent for thin-film microextraction [213]. Ma *et al* [214] prepared single-walled carbon nanomaterials for the analysis of urine samples via MS, utilizing adenosine triphosphate, fatty acids, and peptides as analytes. The limit of detection was found to be 1.0 μM for the urine samples. To summarize and include the remaining work done to date, table 8.1 represents the various NPs used in mass spectroscopy for highly selective analysis.

## 8.4 Conclusion

Mass spectroscopy is a highly reliable, effective, and accurate technique, and there are numerous approaches for mass spectrometry-based imaging. This chapter discusses the use of mass spectroscopy for the characterization, analysis, and use of nanoparticles as matrices for further applications in biomedical fields. The main four nanoparticles being utilized as effective components of biomedical applications have been discussed in detail, i.e., magnetic nanoparticles, bimetallic nanoparticles,

**Table 8.1.** Different nanomaterials and their characterization by mass spectroscopic techniques.

| Nanomaterial | Analytes used | Instrumentation technique | Application | References |
|---|---|---|---|---|
| Silica NPs | Peptides, antiviral drugs | MALDI MS | On plate detection of various particles | [215] |
| Size selected Au-NPs | Peptides, small proteins | MALDI | Mixed with analyte solution as matrix | [216] |
| Au-film | Peptides, nucleotides, nucleosides | MALDI | On plate detection | [217] |
| Au-film coated on nanoporous aluminum oxide layers | Peptides | MALDI | On plate detection | [218] |
| Boronic acid-modified Au-NPs | HRP (horseradish peroxidase) | MALDI | On plate extraction and detection of particles | [219] |
| Au-based Ag NPs | Aminoglycosides | MALDI | Used as a mixture for analyte solution for extraction and detection | [220] |
| Ag NPs | Estrogens (3) | MALDI | Used as a mixture for analyte solution for extraction and detection | [221] |
| Platinum based nanoparticles | Peptides, phospholipids | MALDI | Mixed with the analyte solution as matrix | [222] |
| Titanium dioxide nanoparticles | Mouse brain tissue | MALDI MS | Deposition on tissue surface | [223] |
| Zinc oxide nanowires | Small molecule | MALDI MS | On plate detection | [224] |
| Silanized $Fe_3O_4$ NPs | Peptides | MALSI MS | Mixed with analyte solution as matrix | [225] |
| Pristine carbon nanotubes | Peptides, small proteins, organic compounds and $\beta$-cyclodextrin | MALDI MS | Mixed with analyte solution as matrix | [209, 226] |
| Acidic functionalized fullerene, $C60[(CH_2)_2COOH]_n$ | Peptides | MALDI-TOF MS | Mixed with analyte solution as matrix | [227] |
| Graphene nanoparticles | Amino acids, peptides, acids, fatty acids, polyamines, estrogen, polycyclic aromatic hydrocarbons | MALDI-TOF MS | Mixed with analyte solution as matrix | [210, 228–230] |
| Magnetic graphene and CNT | Amino acids, sucrose | MALDI-TOF MS | Mixed with analyte solution as matrix | [231] |
| P-type h100i (boron) silicon with initiator BisF17 | Clozapine, single cancer cell, biofluids, ketamine, tissues, *N*-desmethylclozapine, norketamine | MALDI | Laser-NIMS | [232, 233] |

metallic nanoparticles, and metal oxide nanoparticles. It can be stated that the design and synthesis of nanoparticles with high selectivity, stability, and efficiency plays a major role in determining their efficacy for biomedical applications. The establishment of molecular imaging techniques was driven by uses for the analysis at the single-cell level for cells and tissues. Techniques including sp-MALDI MS, MALDI-TOF, ICPMS have been discussed briefly in the chapter. For certain applications, nanoparticles can act as a matrix in the mass spectrophotometer instrument thereby ionizing the drugs or its analytes of choice and distinguishing them on the basis of their mass to charge ratios. To date mass spectroscopy has been utilized in many applied and basic sciences and pure samples or complex mixtures in biomedical sciences. The identification of disordered proteins or metabolites for quantitative and qualitative analysis is possible using mass spectroscopy under experimental conditions. MALDI MS and tandem MS are more useful for various applications and are required for full structural identification of complex ligands which are of interest to biomedical applications. MS is currently gaining a lot of attention worldwide for the characterization of nanoparticles. It provides accurate information about the mass of the ligands. The nanomaterials need to be characterized for their functionality and toxicity, therefore the use of mass spectroscopy for the complete in-depth analysis of the film structures with nanometer dimensions as well as the characterization of the surface composition of nanoparticles is found to be highly beneficial. Hence, many potential benefits are possible by utilizing the mass spectroscopy techniques in the future for various biomedical applications and its related areas.

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