A perspective review on green synthesis and characterization of silver nanoparticles (AgNPs) by Ocimum sanctum (tulsi)

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Abstract: In green nanotechnology, an interest is developed among researcher to synthesis nanomaterial. Due to physico-chemical and biological properties of Ag nanoparticles synthesized by bio materials have diverse applications in various fields including biosensors, optoelectronics, drug delivery, and magnetic devices etc. This review emphasis the synthesis of silver nanoparticles by using tulsi *Ocimum Sanctum* leaves under the discipline of green chemistry. Green synthesis method is an eco-friendly method, cost efficient provides a potential to synthesis metallic nanoparticles by different plants. The use of plants for synthesis of Ag nanoparticles has fascinated the researcher to find out the metal uptake & mechanism involved in the reduction of Ag ions into Ag nanoparticles. All the nano particles have a size ranges from 1nm-100nm. To investigate the effect of tulsi and its derivative quercetin regards to the formation of Ag nanoparticles, some physico-chemical conditions are employed e.g., temperature, reactant concentration, pH, and reaction time. The main characterization of Ag nanoparticles generally investigated by various techniques includes UV-Visible spectroscopy, Mass spectrometry, X-RAY Diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) etc. The characterizations reveal that synthesis of Ag nanoparticles by tulsi leaf and its derivative quercetin have the same optical, anti-bacterial and morphological characteristics. So the results demonstrate that quercetin present in tulsi is responsible for the reduction of Ag ion to Ag nanoparticles.

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Introduction

Tulsi (Osmium sanctum) is the most sacred herb of India, also called as Holly basil or queen of herbs. There are present three varieties of Tulsi **a)** Vana tulsi **b)** Krishna tulsi **c)** Rama tulsi. Tulsi is renowned due to its antioxidants properties, restorative power. It contains a number of different compounds with different properties like antioxidant, antibacterial, antiviral, immune enhancing and adaptogenic properties that improve general health. Due to presence of essential or volatile oil, tulsi has specific aromatic fragrance (Chandra, Dwivedi, Arti, & Shinde, 2016).

The predominant cause of mortality is due to chronic effects and mostly addressed by Ayurveda due to its attention on healthy lifestyles. All the herbs used with the Ayurveda, but tulsi is excellent. Scientific research has confirmed that tulsi has many beneficial effects and is preeminent. Tulsi has a unique and mounting evidence that possess all the properties like psychological physical, chemical, biological, and metabolic effects. The side effects of industry pollutants, the chemical stress as well as heavy metals on the human involve in severe exposure to cold, physical restraint and ischemia secured by use of tulsi (Cohen, 2014).

Tulsi has a wide range of applications in pharmaceutical and scientific studies reveal that more than 100 publications had been published during the last decade. Tulsi has many potential pharmaceutical applications like adaptogenic, metabolic, antimicrobial, and adaptogenic, anti-inflammatory, radioprotective, hepatoprotective, immunomodulatory and antidiabetic effects that have been studied in the last decades (Jamshidi & Cohen, 2017).

The chemical effect of heavy metals as well as the industrial pollutants on human body can be protected by using tulsi. Tulsi has prolonged effects on ischemia, physical exertion, and in physical strain. It has metabolic effects by normalizing the blood sugar, lipid level, and blood pressure, and psychological stress by positive way to cognitive function and memory by its anti-depressant and anxiolytic properties. Tulsi provides a broad spectrum of anti-microbial activity, and also involves this activity against a variety of pathogens of human as well as animals (Cohen, 2014).

Nanomaterials were synthesized rapidly during the last decades in the advance field of nanotechnology. The nanomaterials have specific size in (nm) that makes this material more superior and crucial in the area of human activity.

There are present a lot of different methods for the synthesis of nanomaterials, e.g., physical, chemical, biological, condensation, laser ablation method, sputtering method, photochemical method, and green synthesis method. Except to green synthesis method, almost all these methods are potentially dangerous, expensive and more toxic to the environment. Use of biomass, plant extract, microorganism in biological methods could be more beneficial to the nanoparticles synthesis with regards to eco-friendly discipline (Bhattacharya & Gupta, 2005; Sastry, Ahmad, Khan, & Kumar, 2004). The use of natural materials had been adopted by many researchers and reported in the synthesis of nanomaterials. Use of plant extracts, yeast, honey, bacteria, and fungi has widely been selected for the synthesis of (Ag & Au) nanoparticles (Ahmad et al., 2003; Ankamwar, Chaudhary, & Sastry, 2005; Bar & Kr, 2009; Bhattacharya & Gupta, 2005; Chandra et al., 2016; Cohen, 2014; Dwivedi & Gopal, 2010; Gajbhiye, Kesharwani, Ingle, Gade, & Rai, 2009; Gardea-Torresdey et al., 1999; Husseiny, El-Aziz, Badr, & Mahmoud, 2007; Jamshidi & Cohen, 2017; Krishnaraj et al., 2010; Nanda & Saravanan, 2009; Narayanan & Sakthivel, 2008; Parashar, Saxena, & Srivastava, 2009; Pasricha, Singh, & Sastry, 2009; Philip, 2009; Rao, Kotakadi, Prasad, Reddy, & Gopal, 2013; Sastry et al., 2004; Shahverdi, Minaeian, Shahverdi, Jamalifar, & Nohi, 2007; Shankar, Rai, Ahmad, & Sastry, 2004; Sharma et al., 2007; J. Singh, Mehta, Rawat, & Basu, 2018; Singhal, Bhavesh, Kasariya, Sharma, & Singh, 2011; Smitha, Philip, & Gopchandran, 2009; Wang, He, Wang, Zhang, & Tan, 2009).

(Rajakumar & Rahuman, 2011) worked on Ag nanoparticles by using a plant extract of Eclipta prostrata against malarial & filariasis vectors. Mubarak Ali et al. worked on the green synthesis of Ag and Au nanoparticles and also studies their antimicrobial effects against different pathogens (Mubarak Ali, Thajuddin, Jeganathan, & Gunasekaran, 2011). (Sheny, Mathew, & Philip, 2011) worked on the synthesis of silver, gold and silver-gold as bimetallic nanoparticles using dried & aqueous extract of Anacardium occidentale. Prathna et al. worked on Ag nanoparticle synthesis by using Citrus limon aqueous extract and also predict the particle size. (Rao et al., 2013) worked on five different plants e.g., Beta vulgaris subsp., Anethum graveolens, Malva parviflora, Vulgaris, Capsicum frutescens and Allium kurrat and analyzed the bioreduction behaviour for synthesis of Ag nanoparticles. M. parviflora (Malvaceae) has been found to show best results in the synthesis and monodispersity of prepared Ag nanoparticles due to best protecting, reducing and capping properties.

(S. Singh, Saikia, & Buragohain, 2013) synthesized Ag nanoparticles by using aqueous fruit extract Dillenia indica. This fruit is edible and found in the Himalaya's foothills and it is a known due to its antioxidant property as well as a preventive potential against cancer. Ag nanoparticles showed a maximum absorbance of 421 nm under examination of ultraviolet-visible spectrophotometer. Due to simplicity and ecofriendliness of plant mediated synthesized nano particles have great importance. In this review article, we report the simple biological synthesis of Ag nanoparticles by using aqueous leaf extract of tulsi (Holy basil) also known as Osmium sanctum. This plant has been widely used for thousands of years due to its medicinal properties. Tulsi is used in the traditional health system also known as Ayurveda. Tulsi is also known as the Queen of herbs, or mother of medicine in nature. The more significant health effects of tulsi includes dental care, skin care, heart disease and stress, relief of respiratory disorders, fever, asthma, lung disorders. Tulsi has unique potential against the protozoa, bacteria, and fungus. Recently studies reveal that tulsi is significant in inhibiting the carcinogenic cells and also the growth of HIV (Rao et al., 2013).

Green synthesis method is of great interest towards eco-friendly manners for the researchers in the synthesis of metallic nanoparticles. Singhal, Bhavesh et al. worked on the biosynthesis of Ag nanoparticles by using Tulsi leaf extract (Osmium sanctum). The characterization of these biosynthesized nanoparticles was done by using Ultraviolet-visible spectrophotometer, AAS, FTIR, XRD, and TEM. To check the stability of biosynthesized Ag nanoparticles, UV-vis spectrophotometer was used (Singhal et al., 2011).

Metallic nanoparticles have significant applications in the field of industry and scientific research. The unique properties of coinage metals silver and gold nanoparticles have got more attention due to their catalytical, electronic as well as optical properties. Ag nanoparticles have got more attention not only in the industrial applications but also in the fundamental research fields. Ag nanoparticles have been synthesized by different methods like physical, chemical, biological, photochemical methods. In chemical synthesis method, different approaches utilized like thermal decomposition, reduction in different solutions, microwave assisted methods. In chemical synthesis method, different reducing agents of organic and inorganic nature such as $NaBH_4$, ascorbic acid, hydrazine have been used. The silver ions are transformed into Ag nanoparticles by using these reducing agents in aqueous or non-aqueous solvents. The discussed strategies for the synthesis of metallic nanoparticles evenly have been reported effectively, but also suffer from a number of limitations like, harsh nature, chemical hazards to researchers, and potential hazards to environment. So in order to overcome these effects, green synthesis method is more important(J. Singh et al., 2018).

Another study on the green synthesis of Ag nanoparticles has been reported by using Tulsi as a reducing and capping agent. The aqueous solution of AgNO3 and Aqueous leaf extract of Tulsi was used for the rapid synthesis of Ag nanoparticles. These biologically synthesized nanoparticles were characterized by using different physic-chemical techniques like FTIR, UV-vis, SEM-EDX, TEM (Rao et al., 2013). Green synthesis method has gained more attention due to its more attractive features towards non-toxicity, fast, eco-friendly and cost effectiveness. This method is more clean and sustainable for the synthesis of Ag nanoparticles by using tulsi leaf extract (J. Singh et al., 2018).

Synthesis methods of Ag nanoparticles

Synthesis of Ag nano particles is of great interest to the researchers due to their large surface area, significant physico-chemical properties and antimicrobial activities and anticancer agents (AshaRani, Mun, Hande, & Valiyaveettil, 2009). Pure Ag has highest electrical and thermal conductivity and lowest resistance among other metals (Seiler, Sigel, & Sigel, 1988). Nano Ag has significant effects on human body as well as to the environment (Panyala, Peña-Méndez, & Havel, 2008). Many reports on green synthesis have been published using green method due to the capping as well as reducing properties of plants (Gholami, Salavati-Niasari, & Varshoy, 2016; Shahverdi et al., 2007).

Leaves of different plants like tulsi, neem (Verma & Mehata, 2016), and banyan, black tea have been widely used for the synthesis of Silver nanoparticles. Over thousands of years, tulsi leaves used for treatment of many disease due to its medicinal properties. Tulsi has healing property due to presence of essential oils and phytonutrients. Tulsi has significant germicidal, antifungal and antibiotic as well as disinfectant properties that improve the immune system against different viral, fungal and bacterial infections (Banerjee, Satapathy, Mukhopahayay, & Das, 2014).

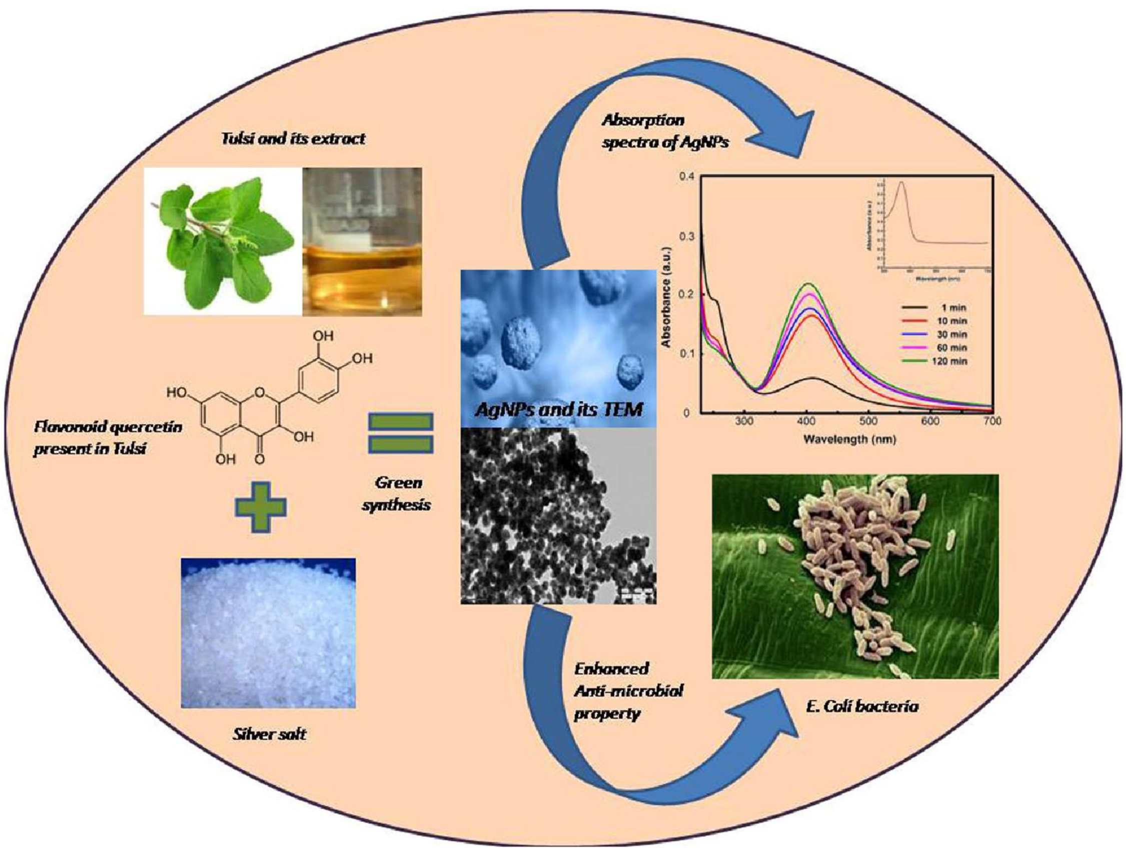

Fig. 1 A schematic diagram for green synthesis method of **Ag** nanoparticles (Jain & Mehata, 2017).

In this review article, we study on the green synthesis method of Ag nanoparticles. The general methodology used for green synthesis of Ag nanoparticles by using various plants has been shown in the diagram 1. But here we emphasize the synthesis of Ag nanoparticles by using tulsi leaves. Firstly, tulsi leaves washed thoroughly with distilled water. The required plant then boiled with water or ethanol to get aqueous plant extract. This plant extract in a fixed ratio is then incubated with desired amount of $AgNO_3$ solution. With the passage of time, the color changed from colorless to brownish due to surface enhanced properties of silver nanoparticles.

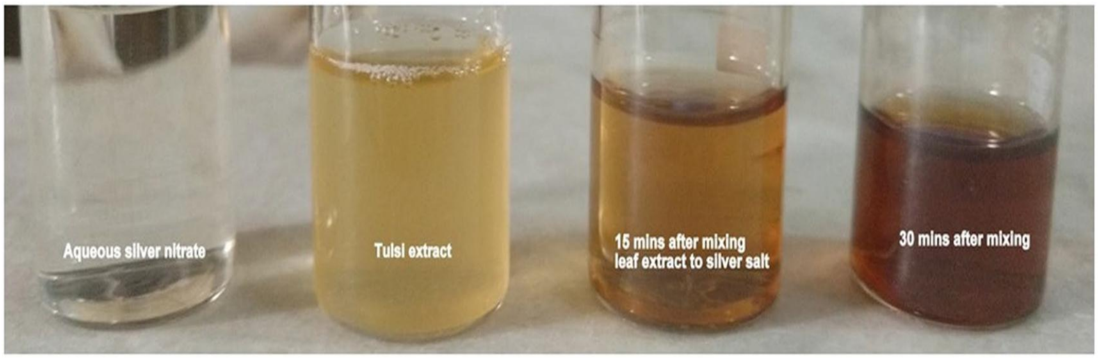

Fig. 2 Change in the colour of the solution with time when plant extracts are added to silver salt (Jain & Mehata, 2017).

The synthesized Ag nanoparticles were characterized by Ultra violet-visible spectrophotometer that showed an absorbance peak at 430 nm. Various techniques were utilized to find out the properties of synthesized Ag nanoparticles (Anjum, Abbasi, & Shinwari, 2016). A number of factors that affect the reduction process of Ag ions to silver nanoparticles e.g., pH, temperature, incubation time, concentration of $AgNO_3$ etc (Rai & Yadav, 2013).

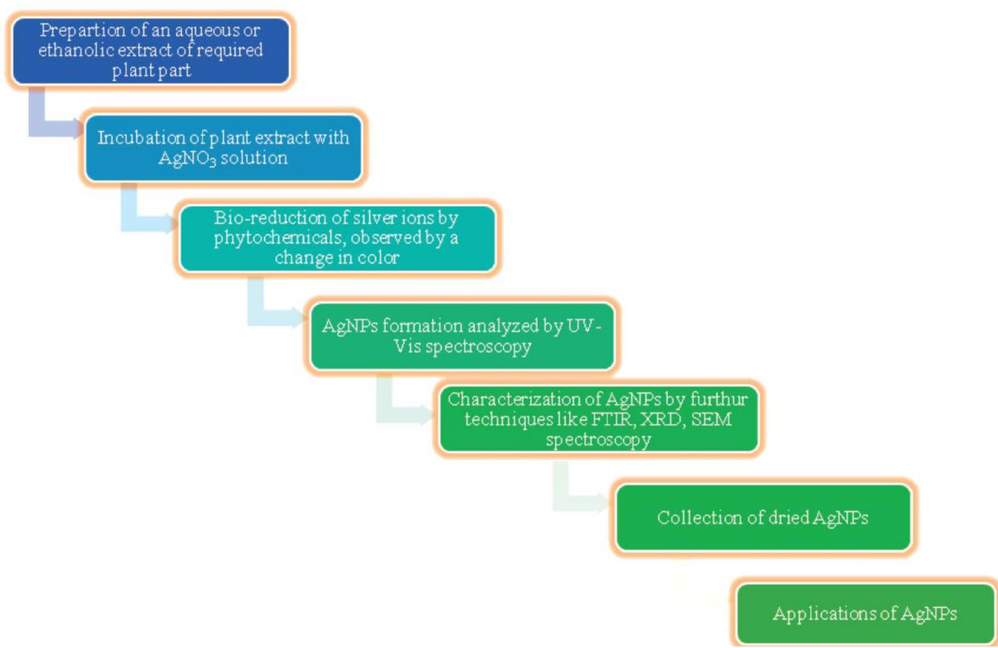

Fig. 3 Various steps involved for green synthesis method of Ag nanoparticles, characterization and applications (Anjum et al., 2016)

Physical conditions for synthesis of Silver nanoparticles:

At regular intervals, time effect by leaf extract was studied about 1, 2, 3 & 4 hours while time of 1, 10, 30 & 60, 120 minutes recorded for quercetin using as a precursor. The difference in the time was recorded as discussed above. The pH of tulsi leaf extract and solution of Silver nitrate was also studied. There was no change in the pH of Silver nitrate solution, while by addition of 0.1N HCl and 0.1N NaOH solution to adjust pH of tulsi leaf extract as well as quercetin solution. The pH of sample was changed from 7-11± 0.2. The variation in the temperature was recorded about 5–35 °C with accuracy ±2°C (Jain & Mehata, 2017). Most exciting and ambient condition for the synthesis of Ag nanoparticles by tulsi leaf extract is the control of temperature. An ambient temperature is responsible for the specific shape and size of Ag nanoparticles. (Song & Kim, 2009) reported the green synthesis of Ag nanoparticles at various temperatures 25, 55 & 95°C. They predicted that with the sudden change in the temperature actually an increase in the synthesis rate of Ag nanoparticles from solution mixture, because the Ag ions converted to Ag nanoparticles.

At 25°C, the transformation of silver ions was almost 60 percent, and became 100 percent at 55°C. By increasing the temperature, the size of silver nano particles decreases from 50nm to 16nm at 25°C-95°C (Anjum et al., 2016).

The size and shape of Ag nanoparticles also depend upon the specific pH. (Sathishkumar et al., 2009) reported the biosynthesis of silver nanoparticles using leaf extract of Cinnamomum *zeylanicum* at various pH of 1 to 11. At lower pH, Ag nanoparticles have ellipsoid shape. While with an increase in pH, spherical shape of Ag nanoparticles were observed. Therefore, with due to change in pH the surface enhanced resonance properties of Ag nanoparticles were also changed (M. Singh, Sinha, & Mandal, 2009).

The concentration of leaf extract is also a factor that is responsible for the synthesis of Ag nanoparticles (Gilaki, 2010). (Bar, Bhui, Sahoo, Sarkar, Pyne, et al., 2009)reported biological synthesis of Ag nanoparticles as well as the effect of concentration of latex and silver nitrate. There was used different concentration of latex 2, 3 and 5 percent. Among these concentrations, the 3 percent was more effective for biological synthesis. The effect of concentration of silver nitrate was also studied. Different concentrations of $AgNO_3$ of 1, 3 and 5 mM was used. The results reported that with an increase the concentration of silver nitrate, the intensity of surface enhanced resonance was also increased (Bar, Bhui, Sahoo, Sarkar, De, et al., 2009)

Characterization of AgNPs:

To understand the size and shape and other characteristics of Silver nanoparticles, a number of physico-chemical techniques have been used. These include SEM, TEM, FTIR, AFM, MALDI-TOF, UV-vis spectroscopy, EDX etc. These analytical techniques are primarily designed for material science to find morphology and other characteristics of metallic nanoparticles, (Ahmad et al., 2003; Anjum et al., 2016; Banerjee et al., 2014; Dwivedi & Gopal, 2010; Kirthika, Dheeba, Sivakumar, & Sheik Abdulla, 2014; Rai & Yadav, 2013).

Different parameters for the determination of specificity of metallic nanoparticles includes particle shape, crystal size, surface area, orientation, pore size, fractional dimension, dispersion and intercalation. These parameters are determined by the techniques above mentioned (Ihmeideh, Al-Omari, & Al-Dababneh, 2010). The formation of Ag nanoparticles is primarily confirmed by UV-Visible spectroscopy (Desai, 2012). The main characteristics values used for maximum absorption find to be in the range of 420-460nm (Yang et al., 2004).

UV-visible spectrophotometer uses a visible and near IR light, so in the visible range absorption and reflection directly affected by the color changed due to reaction involved. The surface plasmon resonance properties of Ag nanoparticles are affected by the size, shape, interparticle size, free electron density. The change in the SPR values can be easily monitored by observing the aggregation of nanoparticles & electron injection through UV-Visible spectroscopy (Anjum et al., 2016).

The crystal size and shape is determined by using X-RAY Diffraction Spectroscopy (Yang et al., 2004). XRD is a widely used for the determination of atomic and molecular structure of a crystal. The incident X-ray beam produced by diffraction from the crystalline atoms in a specific direction. These diffracted beams are used to produce 3D image by determining the angle and intensities of beam. It produces image by the electron density of present within the crystal. The mean position, disorder in the crystal, and chemical bonds can be determined by the electron density (Ealick, 2000).

FTIR is best techniques to identify the capping and reducing agents present in the biomolecule of aqueous leaf extract used for the synthesis of Ag nanoparticles. These capping and reducing agents agent are responsible for the stabilization of Ag nanoparticles (Anjum et al., 2016; Hazarika, Phukan, Saikia, & Chetia, 2014; Jain & Mehata, 2017;

Mubarak Ali et al., 2011). FTIR spectrometer is dominant over a dispersive spectrometer because it collects all the data by means of spectra in a wide range. While dispersive spectrometer collects and measures the intensity in narrow range. The size dependent properties, size dimension & size distribution are not important characterization of Ag nanoparticles but also have significant applications in various fields. To determine the size of Ag nanoparticles, SEM & TEM are generally used, because these techniques provide directly information that associated with the morphology of Ag nanoparticles (Bowers Ii et al., 2009).

TEM has 1000 times more resolution as compared to SEM. The nanoparticles with a size of 0.8nm can be easily magnified and clearly visualized by TEM (Yao, Saeki, & Kimura, 2010). A 3D image of nanoparticles with a size of 0.1nm can be clearly visualized by Scanning Probe Microscopy (SPM) which consist of Atomic Force Microscopy (AFM) & Scanning Tunneling Microscopy (STM). To find the molecular weights of Ag nanoparticles, mass spectrometry is extensively used. Mass spectrometry determines the mass to charge ratio of sample by ionizing it and then separating it into ionic species. The nano particles should not decompose and lose their properties during the ionization process or detection process while in order to find out the molecular weight of nanoparticles. The nanoparticles with higher molecular weights can be easily determined if the array of dimension is large enough. In order to avoid the fragmentation of nanoparticles during ionization and detection, various techniques like MALDI, LDI & ESI are used (Dass *et al*., 2008; Navin *et al*., 2009). The molecules having heavy masses about 300 kilo_ Dalton can be detected by a detector known as time of flight. By joining the MALDI and ESI with TOF are more feasible and advanced detectors for the characterization of silver nanoparticles (Anjum et al., 2016; Kim et al., 2008).

Conclusion:

Nanotechnology is an advanced area of research and innovative approach to generate a material at nanoscale. The association of nanotechnology with reference to green chemistry provides more efficient and feasible nanomaterials. Ag nanoparticles synthesized by biological methods provides eye-catching nanomaterials because it has more potential applications in various fields like medicine, industrial uses, sanitation. This synthesis method is more unique, eco-friendly, feasible, inexpensive, with greater vitality of applications. The biological method is safer than other methods like chemical and physical methods due to the advantage the presence of no residue that harmful to the environment. This method is best alternative to the conventional methods. In biological method, different entities are used to produce Ag nanoparticles. Ag nanoparticles have greater properties than biomedical nanomaterials due to sustainable properties like optical, colorimetric, large size to surface area, physical, and natural properties. Nanoparticles synthesized by biological methods are more applicable with regard to biomedical applications. The Ag nanoparticles can be used in the medicinal transport in future in the area of arrangement. Ag nanoparticles have potential uses in the clinical and pharmacological field. Ag nanoparticles have broad applications including, antifungal, antibacterial, anticancer etc. Ag nanoparticles have distinctive physico-chemical properties like, large surface to volume ratio transfection vectors, binding agents in surgery, in therapeutic compounds. The positive and negative impact of Ag nanoparticles on the human life should be considered before their utilization in various fields. The manufacturing reconstituting of the Ag nanoparticles into different media provide a distinct research field. By modifying the synthesis conditions, like work under someone specialists, use of distinctive engineering techniques and stabilizers, the size, size distribution and shape can be controlled. Synthesis and characterization of Ag nanoparticles is an immense attention in nanotechnology due to its wide range applications in industries.

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