Current Trends

Biosynthesis of Silver Nanoparticles through Medicinal Plants

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Abstract:

Nanotechnology is foreseen to significantly influence science, economy and everyday life in 21st century. Development of new, easy, reliable and eco-friendly technologies helps in endorsing extra interest in the synthesis and application of nanoparticles, which are good and beneficial for mankind. Although, nanoparticles are considered as the discovery of modern science, they actually have a very long history. Ayurvedic metallic preparations, treated with herbal juices or decoction, and exposed for certain quantum of heat as per *puta* (heating grade) system are known in Indian subcontinent since seventh century AD and widely recommended for treatment of a variety of ailments. Such metallic preparations are known as *Bhasma* and are recently proved as nanoparticles.

Today medicinal plants are commonly utilized for the synthesis of nanoparticles. Herbs have shown the ability to interact with metal ions and reduce them to form metallic nanoparticles. The medicinal plant sources for the synthesis of nanoparticle offers several advantages such as best in cost-effectiveness, non-toxic and eco-friendly agent. Biosynthesis of nanoparticles by medicinal plant extracts is currently under exploitation. This synthesis method is more convenient for pharmaceuticals and biomedical applications. Biosynthetic processes of nanoparticles would be more useful, if nanoparticles produced in vitro using plants or their extracts in a controlled method according to their size, shape and dispersity. The utilization of medicinal plants for nanoparticles synthesis is notable alternative in advanced multifaceted approaches.

Key word: nanoparticles, biosynthesis, *bhasmas*,

Introduction:

Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and biotechnology [1]. Modern metal based nanomedicine is a western concept which utilizes metals in very fine particulate forms < 100nm for several purposes including treatment of various sever diseases. This study has revealed some interesting and pertinent information regarding metal based nanomedicines evolving in the present day laboratories which utilize modern biomolecular and biochemical techniques. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology [2]. Concept of reduction in particle size of metals is prevailing since Charaka Samhita (1500 BC). A metallic preparation, *Lauhadi Rasayana* (a rejuvenating formulation in which iron is first ingredient) has been mentioned in Charaka Samhita which is advised to

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prepare by heating iron up to red hot and quenching immediately in some liquid media until flakes of iron become in fine powder form [3]. It can be interpreted that, this procedure helps in impregnating bio-molecules of herbal media on the surface of fine metal particles and thus creates nanoparticles of iron. Nanoparticles are present abundantly in human body even at cellular level and thus it is assumed that nanoparticles may cure sever diseases, chronic diseases and even genetic disorders. On the basis of researches performed uptill today, it can be claimed that Nanotechnology has ability to work at these subtle levels, to generate new structures with new molecular organization [4]. *Bhasmas* (Incinerated metallic preparation) which are unique Ayurvedic metallic/mineral preparations, treated with herbal juices or decoction, and exposed for certain quantum of heat as per *Puta* system of Ayurveda are known in Indian subcontinent since seventh century AD and widely recommended for treatment of a variety of ailments . *Bhasmas* are claimed to be biologically produced nanoparticles prescribed with several other medicines of Ayurveda.[5]

Table no.1:- Different medicinal plants used for the synthesis of nanoparticles of metals

Medicinal plants	**Nanoparticles produced**	**Size**	**References**
Amaranthus spinosus Linn. Leaf	Gold (Au)	10.74 nm	Das R K et al., 2012
***Gloriosa superba* Linn.**	Copper (CuO)	5–10 nm	HR Naika et al., 2015
Cinnamomum camphora Nees. & Eberm. Leaf	Palladium (Pd)	3.2 to 6.0 nm	Xin yang et al., 2009
Fusarium oxysporum	Silica (SiF62-) and Titania (TiF62-)	-	V Bansal et al., 2005
Ocimum sanctum Linn. Leaf	Platinum	2-12 nm	Soundarrajan C et al., 2012
Zingiber officinale Roscoe. *Root*	Zinc oxide (ZnO)	30-50nm	L. F. A. Anand Raj et al., 2015
Ocimum sanctum Linn. Leaf	Iron oxide (Fe2O3)	47 nm	Balamurughan M G et al., 2014
Ocimum sanctum Linn. Leaf	platinum (Pt)	23 nm	Soundarrajan C et al., 2012
Vitus vinifera Linn.	Lead (Pb)	661nm	Pavani et al., 2012

Table no.2:- Different medicinal plants used for the synthesis of silver nanoparticles

Medicinal plants	**Nanoparticles produced**	**Size**	**References**
Cinnamomum camphora Nees. & Eberm. Leaf	Silver (Ag)	55 to 80 nm	Huang et al., 2007
***Piper longum* Linn. fruit**	Ag	46 nm	NJ Reddy et al., 2014
***Allium sativum* Linn.**	Ag	4 .4 ± 1.5nm	White II et al. 2011
***Achyranthus aspera* Linn.**	Ag	20-30nm	Daniel et al. 2012
***Carica papaya* Linn.**	Ag	15nm	Jain et al. 2009
Coleus aromaticus Lour.	Ag	40–50 nm	Vanaja et al.2012
***Citrullus colocynthis* Linn.**	Ag	31nm	Satyavani et al. 2011
***Datura metel* Linn.**	Ag	16 to 40 nm	Kesharwani et al 2009
***Desmodium triflorum* (L) DC.**	Ag	5–20 nm	Ahmed et al.2011
***Glycyrrhiza Glabra* Linn.**	Ag	20 nm	Dinesh et al.2012
***Hibiscus cannabinus* Linn.**	Ag	9 nm	Bindhu et al.2012
***Piper betle* Linn.**	Ag	3-37 nm	Mallikarjuna et al.2012
***Piper nigrum* Linn.**	Ag	5 - 50 nm	Garg, S. 2012
***Solanum xanthocarpum* Linn.**	Ag	10 nm	Amin et al.2012
***Emblica Officinalis* Gaertn.**	Ag	10 to 20 nm	Ankamwar *et al.,* 2005
***Aloe vera* (L) Burm.F.**	Ag	15.2 nm ± 4.2 nm	Chandran *et al.,* 2006
***Syzygium cumini* (L) Skeels. seed**	Ag	92 nm	Kumar et al 2010

Methods and methods:

Research papers published on nanoparticles were collected from internet. Selected information was interpreted regarding the methods for the synthesis of nanoparticles, characterization and uses at different fields. Biological sources such as bacteria, fungi, yeasts, algae and plants are used for the synthesis of nanoparticles. But now a day's plants are very commonly used for the synthesis of nanoparticles. The most important application of silver and silver nanoparticles in the medical industry includes preparation of topical ointments to prevent infection against burnt and open wounds. These particles have diverse applications both in vitro and in vivo [10]. A number of medicinal plants are being currently investigated for their role in the synthesis of nanoparticles. For general understanding, synthesis of silver nanoparticles has been given below.

1. Preparation of medicinal plant extract (aqueous):

Collect fresh plant material (leaves/ root/bark etc.) of selected medicinal plant. The plant material should be mature, undamaged and diseased free. Wash the plant material thoroughly with sterile distilled water. Take the 25 gm of sterilized plant material cut into small pieces and put in a 500 ml conical flask containing 100 ml sterile distilled water, boiled it for 5 min and then filter. The filtrate should be stored at 4^0C for uses.[11]

2. Synthesis of nanoparticles (silver):

Add 1 mM silver nitrate to the plant extract in a conical flask and centrifuged at 18000 rpm for 25 min. Heat the supernatants at 50-95^0C. Changes will occur in the colour of the solution during heating process within 10-15 minutes. The colour changes indicate the formation of silver nanoparticles.[12] Figure 1 shows that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface Plasmon vibrations. As the extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from colourless to yellowish brown due to reduction of silver ion which indicated formation of silver nanoparticles.

3. Characterization of nanoparticles:

Absorbance spectroscopy is used to determine the optical properties of a solution. A ray of light is sent through the sample solution and the amount of absorbed light is measured. When the wavelength is varied and the absorbance is measured at each wavelength. The absorbance can be used to measure the concentration of a solution by using Beer-Lamberts Law. The examination of nanoparticles shows that the optical properties are much more complicated. For instance, the measured absorbance spectrum does not necessarily show the actual absorbance but the extinction of the light is both the absorbed and the scattered light from the particles. These wave lengths arise due to the surface Plasmon resonance of the particle. Figure 2 shows the UV-VIS spectra recorded; broadening of peak indicated that the particles are polydispersed.

Scanning electron microscope (SEM) analysis is employed in characterization of size, shape & morphologies of formed nanoparticles. SEM gives high-resolution images of the desired surface of a sample. Scanning electron microscope works on same principle as an optical microscope, but it measures the electrons scattered from the sample rather than photon. Because electrons can be accelerated by an electric potential, the wavelength can be made shorter than the one of photons. This makes the SEM capable of magnifying images up to 200.000 times. At the same time it is possible to achieve high resolution pictures of the surface, making the instrument very useful in determining the size distribution of nanoparticles. (Figure 3)

AFM (**Atomic Force Microscope**) is an instrument capable of measuring the topography of a given sample. A nano-sized tip attached on a cantilever is traded over the sample and a 3D image of the sample topography is generated on a computer. The advantage of the AFM over SEM is the ability to make topographical measurements for detection and investigation of the size and shape of silver nanoparticles in three dimensions. The AFM generally measure the height of silver nanoparticles. (Figure 4)

DLS (**Dynamic Light Scattering**) technique uses light to determine the size of particles in a solution. Light at a given frequency is sent through the solution from a laser. When the light interacts with the moving particles in the solution and is scattered, the frequency of the light is also changed. This change of light frequency is directly related to the size of the particles in the solution; the smaller the particles, the greater the shift in the light frequency. This difference in the light shift is used to determine the size of the particles in the solution. DLS is capable of measuring particles in the size range from a few nanometers to a few micrometers. It is therefore applicable for determining the size of silver nanoparticles. (Figure 5)

FTIR (**Fourier Transmission infrared spectroscopy**) is a chemical analytical method which measures infrared intensity v/s wavelength or wave number of light. It used to analysis of possible bio molecule and also bonding interaction between themselves. IR spectroscopy detects the vibration characteristics of chemical functional groups of the sample. When an infrared light interacts with matter, chemical bonds will shows stretch, contract and bend form. This chemical functional group tends to adsorb infrared radiation in a specific wave number range of the structure of the rest of the molecule. The silver nanoparticles synthesis, FTIR data measures interaction between Ag salts and proteins molecules, which accurate for the reduction of silver ions and stabilization of Ag NPS formed. (Figure 6)

XRD (**X-Ray Diffraction**) is a technique to used go study phase composition of a sample, crystal structure, texture or orientation. The principle of XRD is that the X-rays are passed through a material and the pattern produced give information of size and shape of the unit cell. The atoms are crystal in structure arranged in a periodic array and thus can diffracted light at different angle. When X-ray passing through a crystal it produces a diffraction pattern, that diffraction gives the information about the atomic arrangement within the crystals. In silver nanoparticle XRD gives phase structure and purity of the particle. Figure 7 shows the broadening of peak indicates uniform distribution of silver nanoparticles reveals that these particles would help for the availability of the drugs throughout the system (body). The biosynthesis of silver nanostructure by employing medicinal plant extract was further demonstrated and confirmed by characteristic peaks observed in the XRD image.

Nanoparticles as per Ayurveda point of view:

Ayurveda recommended metallic preparation with extracts of herbal juices known as *bhasma* for the treatment of so many diseases. These metal based drugs are found highly effective than their original metallic forms. *Bhasmikarana* is a very systematic and elaborate step-wise procedure to convert metal form its zero valent state to a form with higher oxidation state. During this process the toxic nature of the metal and its oxide is fully destroyed while rendering the metal oxide with high medicinal value. [13] Before *Bhasmikarana* metals/minerals are advised for subjecting some procedures known as *Shodhan.* During *Shodhana* process metals are repeatedly subjected for heating with herbal juices which make them brittle, removes some unwanted properties and helps to impregnate molecules of herbal media on the surface of metal/mineral. The second step includes mixing the transformed metal with herbal decoction and introducing it to fire to turn it to ashes, a process called incineration. In this process, the metallic drug is converted from a heavy, hard and rough structure to light, soft and smooth powder and the macro size particles are reduced to their 'nano' form (usually 10-50 nm) [14]. The metal is combined with herbs which help in assimilation and delivery of the ingredients into the human body [15].

Discussion:

In biologically created nanoparticles, metals are combined with herbs which help in assimilation and delivery of the ingredients into the human body [16][17]. Counterparts are stable over longer period of time, require lower dosages, are easy to store and have sustainable availability [18]. Synthesis of nanoparticles using biological entities has great interest due to their unusual optical [19], chemical[20], photoelectro-chemical[21] and electronic properties[22]. The synthesis and assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable 'green chemistry' procedure [23-24]. Due to distinct properties of silver nanoparticles such as good conductivity, chemically stable, catalytic activity, surface enhanced Raman scattering and antimicrobial activity have attracted and demandable research of interest in the field of nanotechnology. In this era silver is use as antimicrobial agent. Recent focuses towards silver nanoparticle synthesis for increasing the treat of antibiotic resistance, caused by the misuse of antibiotic.

Herbal substances are act as chelating for biosynthesized nanoparticles and because of such property the drug gets easily absorbed in the body and they are target drug delivery and easily eliminated out of body. Herb reduces the toxicity of metal, converting it to herbo-metallic form, enhancing its therapeutic quality so that it is effectively used by the

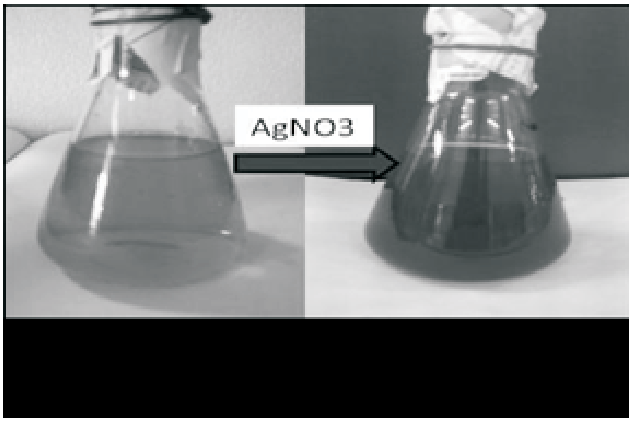

Fig 1: Formation of silver nanoparticles

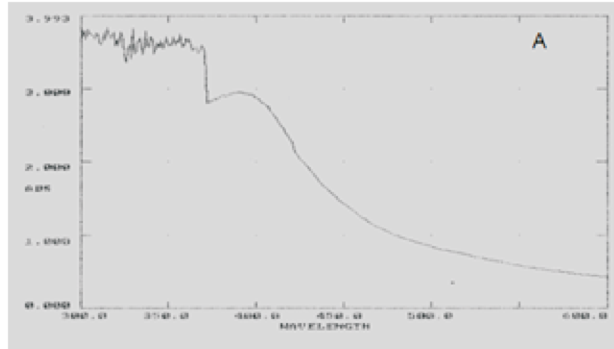

Fig. 2: UV-Vis Spectra of reduction of Ag ions to Ag nanoparticles

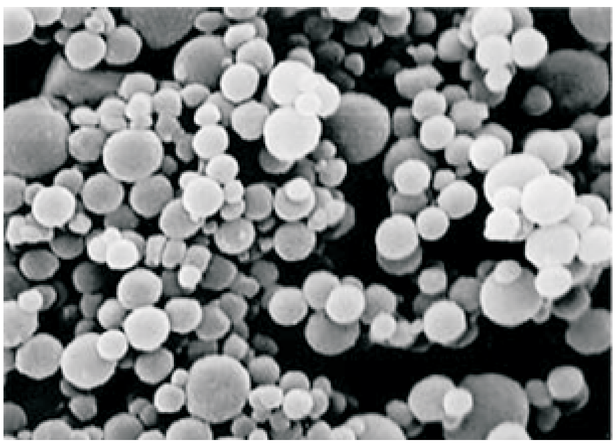

Fig. 3: SEM shows high-resolution images

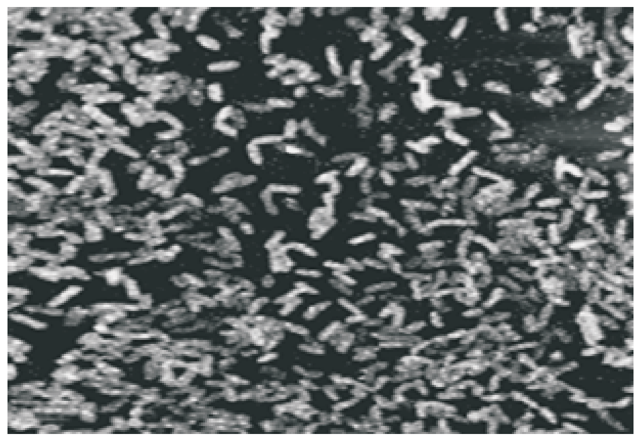

Fig. 4: Topographical measurements by AFM

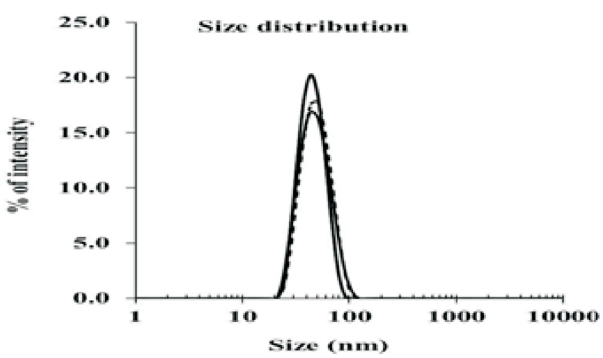

Fig. 5: DLS determining the size of silver nanoparticles

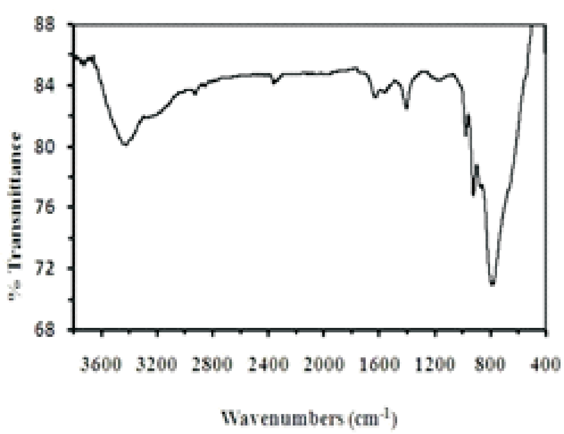

Fig. 6: FTIR measures infrared intensity v/s wave number

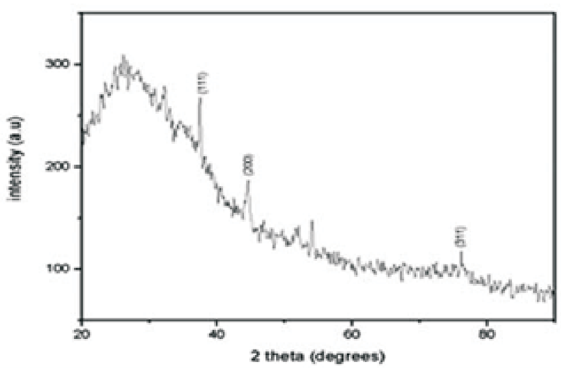

Fig. 7. XRD shows uniform distribution of silver

body. Acharya Charaka says '*Samskaram gunanthardhanam'* by this the quality of herbal drugs is attributed to mineral drugs.

Conclusion:

Form above study its concluded that green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis as in this method there is no need to use high pressure, energy, temperature and toxic chemicals. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic field will make revolutionary growth in discoveries in related filed. The characterization analysis proved that the particle so produced in nano dimensions would be equally effective as that of antibiotics and other drugs in pharmaceutical applications. It is a new and emerging area of research in the scientific world, where day-by-day developments is noted in permitting a bright future for this field.

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