

ORIGINAL ARTICLE

Green-Synthesis of Silver Nano-particles by Using *Cyamopsis tetragonoloba* L. Seeds and their Characterization

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ABSTRACT

Silver metal is the most fascinating and potential metal used to prepare Nano-particles which are used for multiple purpose like nano medicines. Antimicrobial activity of silver nano-particles by using *Cyamopsis tetragonoloba* plant seeds were studied against *E.coli* and *Bacillus subtilis* bacteria. Reduction of silver nitrate take place in the presence of *Cyamopsis tetragonoloba* Seed extract which forms Silver Nano particles. Aqueous solution of Silver Nitrate (1mM) was prepared for the synthesis of silver Nano-particles and mix with the methanolic extract of *Cyamopsis tetragonoloba* seed extract under normal condition. This solution was maintained in dark room for 48hrs, after that colour change was observed dark green into brown colour. This colour change confirmed the development of silver nano particles. Characterization of formed silver nano-particles were done by UV-Visible spectroscopy. The UV-Vis bands showed maximum peak at 430 nm and red shift with time indicating the increase in particle size. Further analysis were done by using XRD, SEM and TEM. The optimum size and shape of the particles was found to be 25-100 nm and in crystalline dimensions. FT-IR shows the stretching and bending of present functional group. NMR was done for showing absorbance of Electro magnetic Radiation.

**Key words:** Silver nano particles, *Cyamopsis tetragonoloba*, *E.coli*, *Bacillus subtilis*

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INTRODUCTION

Silver Nano-particles are beneficial in various fields like medicine, research, diagnosis, food, health care and industries. With the advance investigation in Nano Science change the way of human life style. For the development of Nano-particles green processes evolved an important tool in nanotechnology. Nano biotechnology is a field that has been developed rapidly, showing a great influence in all domain of human life. There are numerous applications for the synthesis of silver nano-particles like reduction method, chemical synthesis and, and biological method. The biological synthetic method by using plant extracts has been used widely because it is ecofriendly, and economically cheap method as compared to all other existing methods. Biological method is showing great attention because of using bacteria and fungi for synthesis of metal nano particles.

Nano Biotechnology is having great influence on human health and their approaches are growing enthusiasm in the medical sciences mainly in medicine, microbiology and biotechnology [3]. Many metals are available for the synthesis of Nano-particles like copper, gold and Silver. Among all these metal NPs, Silver Nano-particles are very encourage able and can be used in different fields like Microbiology, Biotechnology, Bio-medicine. Metallic Nano-particles ranges between 1 to 100nm have profound interest in studies. Genuinely Silver is important for research because it does not create feasible environment for microbial resistant in to the host cell. That why Silver metal is intermixed with materials like food stuffs, packing material and in fabrics. There are lot of application of silver Nano-particles so that several silver bio-synthesis based amalgam have been developed [12]. Silver Nano particle is the centre of attraction for researcher because of their wide importance in medical science [9]. Silver nano-particles have remarkable importance and applications in many meadow like genetic engineering, Biotechnology,

Biosensor, Bio medicine and Microbiology. [1]. Silver metal proves to be very harmful and has toxic nature against a variety of microorganisms. Silver Nano-particles have anticancer, antiviral, antibacterial and anti-inflammatory properties, and proves as very vigorous agent in the fields of biology and medical science. Green synthesis of Metal nano-particles is better choice over other method used like synthetic, chemical method or micro-organisms methods because it is economically cheap, and environmental friendly. Medicinal plants have been used since ancient to cure many diseases because of their medicinal values. Therefore, Medicinal plants and their products are extensively studied in Chemistry, Bio-medicine, Ayurveda, Antimicrobial and essential oils. [8, 9]. Natural products like Lignin, tannin and Phenols obtained from medicinal plants used in Ayurvedic medicine as these medicinal plants are used in biological, biotechnological, microbiological and pharmacological activities [6]. In current years, scientists and researchers are keenly curious in studying the wide applications of these medicinal plants by analyzing its vital ingredient liable for their remedial effects. Guargum (*Cyamopsis tetragonoloba* L.) is one of the vital medicinal plant which have lot of health benefits. *Cyamopsis tetragonoloba* L. has medicinal qualities because research on this plant proves that it is anticancerous, antimicrobial, antioxidant, anti diabetic and very efficient in cure of cardiovascular disease and to control obesity. The main aim of this study is to synthesize and characterize AgNPs using the methanol and Ethanolic extract of *Cyamopsis tetragonoloba* seed.

## MATERIAL AND METHODS

### Preparation of Plant Extract

The Seeds of *Cyamopsis tetragonoloba* Beans were purchased from the Market. The beans were then absolutely washed with tap water and then with distilled water, After that beans were dried under shade by covering with transparent cloth. The beans then crushed with the help of Mortar and Pestle and seeds were separated out. The dried seeds parts were integrated into thin powder form and stored in air tight container which was stored for further used for solvent extraction.

### Soxhlet Extraction Procedure:

**Soxhlet Extraction** is a laboratory apparatus. A Soxhlet extraction is used when the intended compound has a finite solubility in the solvent. About 40 gm of *C. tetragonoloba* bean powder material were being uniformly packed in to a thimble and move in Soxhlet extraction (fig.1). It was depletable extracted with 200 ml Methanol and Ethanol solvents separately for the duration about 48 hour or 24 cycles until the solvent (Methanol and Ethanol) in the siphon tube of an extract become colour less. After completion of this process Seed extract was filtered with the help of Whatman filter paper. The bean extract were used against *E.coli* (Gram -ve) and *Bacillus subtilis* (Gram +ve) Bacteria for MIC and MBC. The residue left was dried over anhydrous  $\text{NaSO}_4$  to remove left residue of alcohol. Then Seed extract kept in refrigerator at  $4^\circ\text{C}$  for detection of antibacterial activity and to examine their physical and chemical property.

### Green synthesis of silver nano particles-

Silver nitrate used in synthesizing Silver Nano-particles was purchased from Pfizer, India. In four different conical flask 50 mL, 1 mM solution of silver nitrate was prepared. In each 50 mL of silver nitrate solution maintaining its concentration at 1 mM, 1ml, 2ml, 3ml and 4ml Plant Seed Extract were added. Silver nano-particles were synthesized at various concentration of  $\text{AgNO}_3$  like 1mM, 5 mM, 10mM and 15mM) adding plant extract concentration 1ml, 2ml, 3ml and 4ml. All these Flask of silver nano-particles synthesize was incubated in a dark cubicle to prevent photo-activation of silver nitrate at 298K. Reduction chemical reaction take place in which  $\text{Ag}^+$  to  $\text{Ag}^0$  oxidation state was confirmed by greenish colour of the solution changes into dark brown colour. After that, Synthesis of SNPs was confirmed by using UV-Visible spectroscopy.

**Bactericidal activity of Silver Nano particles-** Antimicrobial activity is a procedure, in which killing of microorganisms and stops their development take place. The in vitro antimicrobial testing of the silver Nano-particles prepared from purified extract of *C. tetragonoloba* beans tested and established against the selected *E.coli* and *Bacillus subtilis* bacteria by using dilution method and minimum inhibitory concentration (MIC) methods. This method is used in a number of different samples to calculate the number present of micro-organisms in a given volume. This can be performed by using the serial diluting the bacterial population. This method provides statistically accurate information [6]

### Principle of Techniques used for Characterization of Silver Nano-particles-

#### UV-Visible Spectroscopy

UV-visible spectroscopy is significant technique used for evaluation of silver nano-particles in different solvent. Silver nano-particles surge to plasma resonance absorption band which arise due to combined vibration of metal nano-particles in oscillation with the light wave. The prepared sample is dissolved de-ionized water, in which metal nano-particles can be synthesized by reduction method where metal ions

use plant extract components. In green synthesis of nano-particles plant extract is considered as natural reducing agent.

### Fourier Transform Infra Red Spectroscopy

FTIR is a Infra- Red spectrophotometer which is used to obtained absorption and emission of spectrum at particular wavelength of sample i.e. solid or liquid. This highly efficient technique is based on OMNI-sampler attenuated total reflectance (ATR) accessory . In this technique the sample was placed on the sample holder where infra red radiation continuously worked with applied constant pressure. A sample absorb the radiation over the wave number dimensions from 4000cm<sup>-1</sup> to 450cm<sup>-1</sup>.The result shown on computer screen in the form of peaks which were further used for data analysis

### Scanning Electron Microscopy

Scanning electron microscopy model JSM7610F was used for analysis. It works on the principle of scanning the surface by use of electron beam. These electron beam make interaction with the metal atom present in the sample. Signals detected on the combination of electron beam and position of beam which create a image and recorded on the computer screen.[11]

### Transmission Electron Microscopy-

This works on the principle where a beam of electrons passed through the prepared sample and absorbed into consistent beam by the use of the condenser lens. The electron beam then strike the sample and some parts of it are transmitted through it and create a image which depends on the sample thickness in which electron beam passes [4].

### X-Ray Diffraction

X-Ray diffraction works on the principle of constructive interference of monochromatic radiations. X-ray detector determined the chemical distribution of synthesized silver nano-particles and used for diagnosis of crystalline structure of the compound. It gives clue on unit cell dimensions. Cathode rays are responsible for generation of X-rays, which filtered and leads to monochromatic radiation, assemble at one point, and assisted through the sample [14]. When incident rays interacts with the sample constructive interference generated.

### Nuclear Magnetic Resonance-

Nuclear magnetic resonance spectroscopy or magnetic resonance spectroscopy (MRS), is a technique used to analyze magnetic fields around atomic nuclei and each atomic nuclei have spin. when an electric current is applies these spin rotate clockwise and anticlockwise When magnetic field is applied energy transfer take place lower energy level to higher Energy level. It is useful in determining the molecular structure of the compound. This technique is based on the absorption of electromagnetic radiation.

## RESULT AND DISCUSSION

**UV-visible spectroscopy analysis-** A surface plasma resonance spectrum of silver nano particle was obtained at 437nm after color changee Greenish to brownish color. The Nano-particles were reviewed at the wavelength ranging from 250-550 nm using Systronic Spectrophotometer. The solution was examined at intervals of 45 nm and all position of peaks were detected. The highest value peaks of the UV-Visible was recorded at 430 nm as in Fig-1.

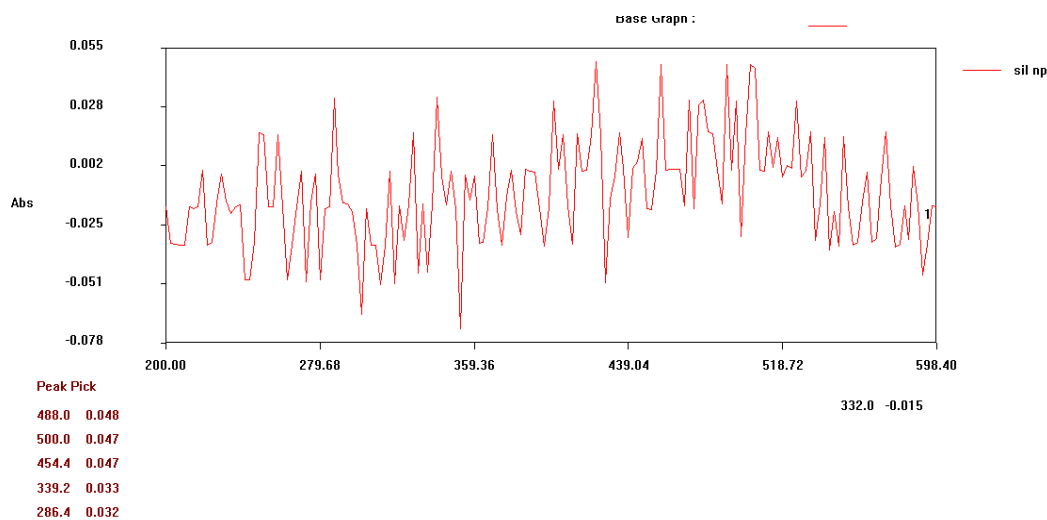


Fig-1, UV-Vis Spectroscopic Peaks and Pick points

**XRD spectrum analysis-** The chemical composition of bio synthesized silver nano-particles was revealed by XRD spectrum. It gives idea of crystalline structure and tells about the chemical bonding and non covalent bonding between the atoms. In this study SNPs size range is  $2\mu\text{m}$  shown by XRD(Fig-2).

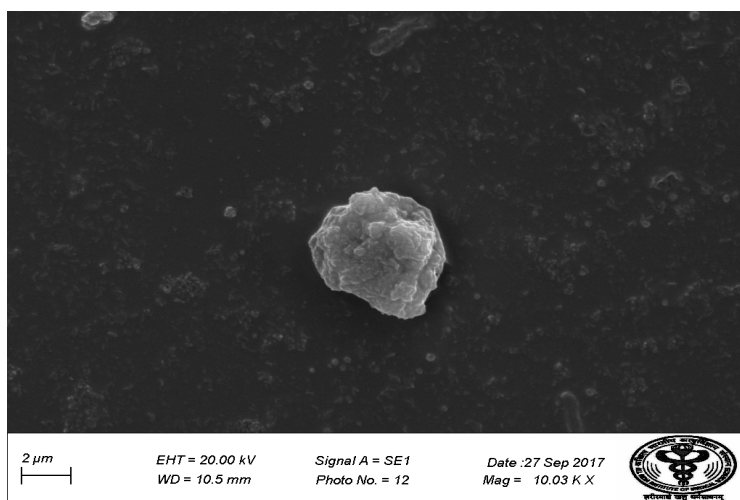


Fig.2- Showing XRD spectrum

**FT-IR spectroscopy analysis-** The Infra Red spectrum of silver nano-particles shown bands at  $3434.72\text{ cm}^{-1}$  and  $4000.20\text{ cm}^{-1}$  (phenolic O-H stretching vibration). The peaks at  $2080.70\text{ cm}^{-1}$  indicates C-H stretching of methylene group. The bands at  $1637.66\text{ cm}^{-1}$  states primary amines. The bands at  $1403.18\text{ cm}^{-1}$  state C-H alkenes and bands at  $1123.77\text{ cm}^{-1}$  indicates the presence of C-O stretching of alcohol,  $1015.19\text{ cm}^{-1}$  state carboxylic acids,  $697.91\text{ cm}^{-1}$  represent esters. The phytochemicals like flavanones, quinones, Lignin, Tannin and Phenols present in *Cyamopsis tetragonoloba*, are responsible for reduction of silver ion into silver metal. Secondary metabolites like terpenoids having functional group of alcohols, ketons, aldehydes and carboxylic acid present in *Cyamopsis tetragonoloba* seeds may be considered as the significant observation (fig-3).

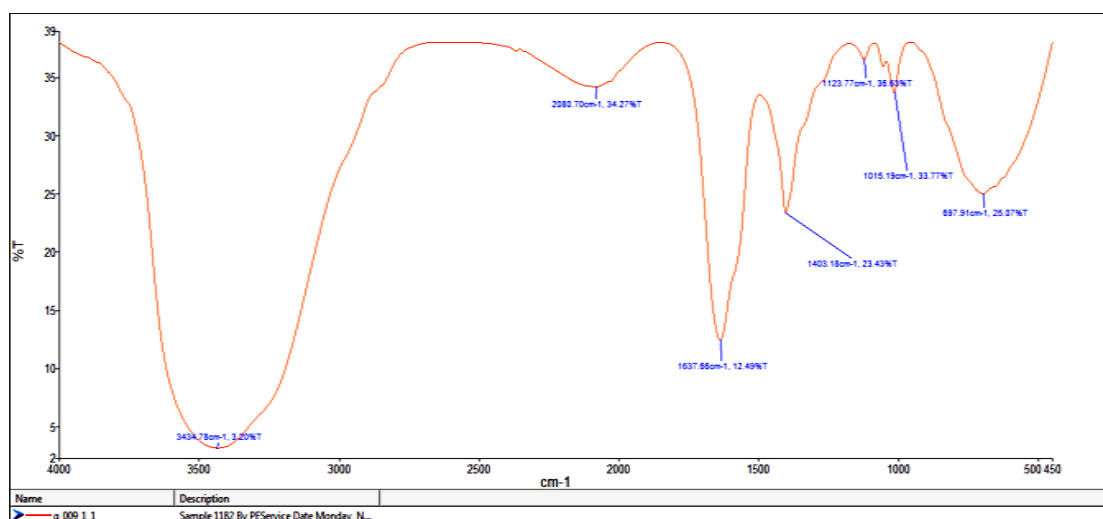


Fig-3, FT-IR showing various functional group stretching

**Scanning Electron Microscopy (SEM) analysis-** A small drop of sample taken on slide then make a thin layer over glass slide, the residue were removed with the help of blotting paper then slide is kept in hot air oven to dry for 15 min. For getting clear image the sample need to be coating with metal. In this study, silver metal is used for coating because it is good conductor of electricity. SEM analysis determine that the particle range is between  $2\mu\text{m}$ - $5\mu\text{m}$ . The SEM result shown in below Fig-4.

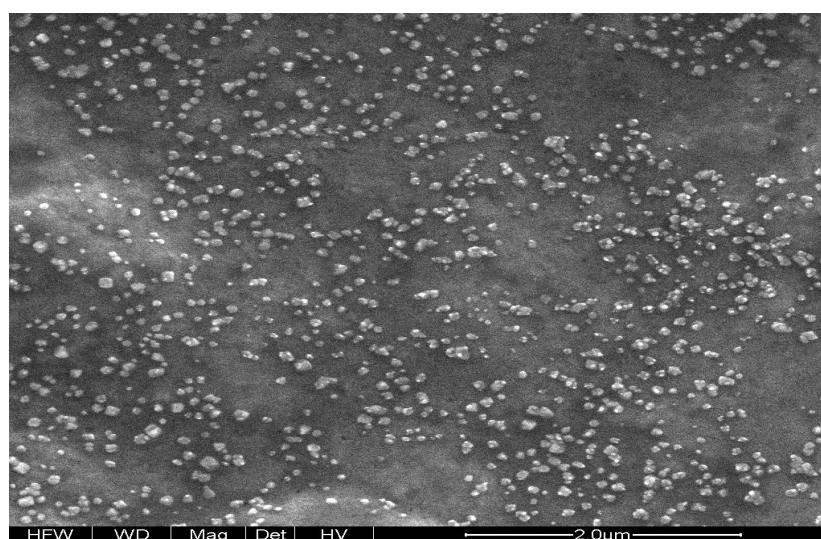


Fig-4, SEM, showing silver Nano-particle size in Between 25nm to 100 nm

**Transmission Electron Microscope(TEM) analysis-** When Thin layer of sample applied TEM generate a beam of electrons through the sample to produce a magnified image of the compound. The electricity provide the power to cathode rays as it develop a beam of electrons which works in alike to the beam of light works in an optical microscope Fig-5(a) and(b).

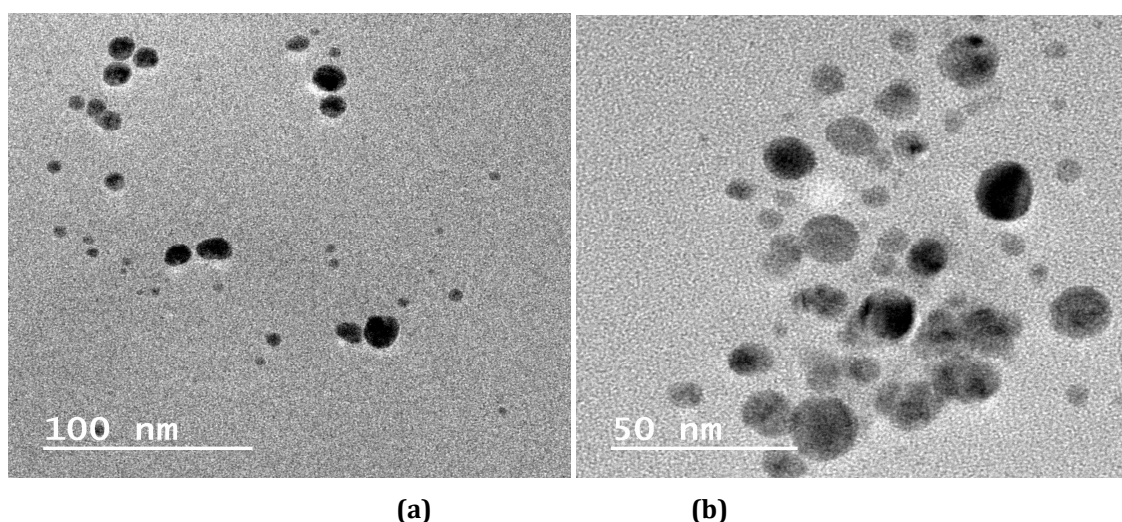


Fig-5, TEM- Showing Silver Nano-particle size 50nm and 100nm

**Nuclear Magnetic Resonance(NMR) analysis-** It generate the powerful magnetic field that is ten thousand times greater than the earth's magnetic field. When samples were placed between magnetic field and exposed to radio waves, spectrometer transmit and receive the radio waves helps in NMR measurement. Data processing seen on the computer screen as shown in the below figure 6.

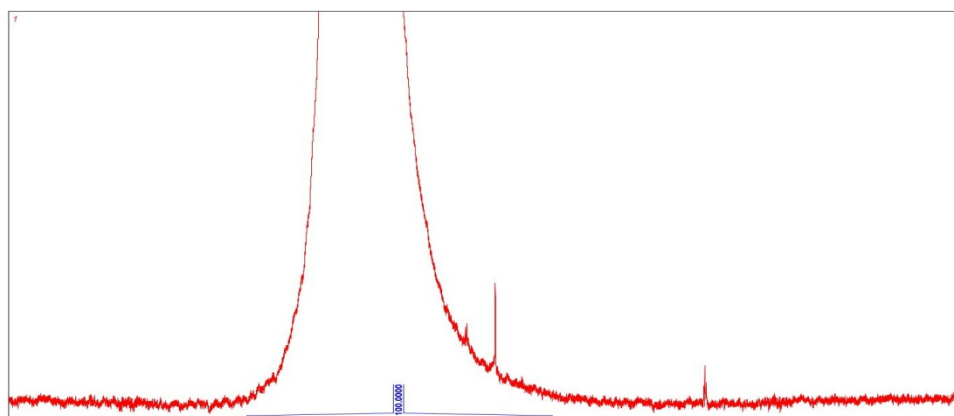


Fig-6, NMR- spectrum showing absorption of electromagnetic radiation

**Antibacterial activity of Silver Nano particles-** Antibacterial activity of silver Nano-particles using *Cyamopsis tetragonoloba* seed extract shows effective minimum inhibitory zone against *E.coli* and *Bacillus subtilis* (Fig-7). Minimum inhibitory zone in *E.coli* is 10-15mm while in *Bacillus subtilis* is 9-12mm clearly mentioned in Table-1 and Table-2.

**Table-I: Antimicrobial activity of different extracts of *Cyamopsis tetragonoloba* against pathogenic microbes**

S.No.	Solvent	Plant Part	Inhibition zone in (mm) against pathogenic microbes after 24 hrs incubation ( <i>E.coli</i> )		
			0.75mg	0.5mg	0.25mg
1	Silver Nano particles	Seed	15	12	10

**Table-II: Antimicrobial activity of different extracts of *Cyamopsis tetragonoloba* against pathogenic microbes**

S.No.	Solvent	Plant Part	Inhibition zone in (mm) against pathogenic microbes after 24 hrs incubation ( <i>Bacillus subtilis</i> )		
			0.75mg	0.5mg	0.25mg
1	Silver Nano particles	Seed	12	10	09



(*E.coli*)

(*B.subtilis*)

**Fig-7: Antibacterial activity of SNPs against *E.coli* and *Bacillus Subtilis***

**CONCLUSION**

The present invention of silver nano-particle bio-synthesis is a low-cost, green, eco-friendly approach, capable to producing good minimum inhibitory zone against *E. coli* and *Bacillus subtilis*. These biologically synthesized silver nano-particles (*Cyamopsis tetragonoloba*) were found suitable for inhibitory effect on Obesity, diabetes and dyspepsia.

The synthesized silver Nano-particles had great influence on human health. It was found that the at higher temperature, larger size of Silver nano-particles are formed and rate of reaction also slower down. Aqueous solution of Silver nitrate was intermixed with *Cyamopsis tetragonoloba* seed extract, then bio-synthesis of AgNPs were confirmed by colour change of solution. UV-Vis spectroscopy shows that 430 nm

height peak point. FT-IR confirmed the presence of variable functional group in the form of stretching and bending structures. These functional groups accountable for the formation of various organic compound and these organic compounds present in the seeds serve as an effective reducing agent. The NMR result shows the behavior of electromagnetic radiation towards organic compounds. These bio-synthesis silver nano-particles can potentially be used for different medical purpose like to diagnose disease and their treatment.

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