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The Green Synthesis, Characterization of Silver Nano Particles by Methanol Extract of Marine Sponge – Spongiatosta

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ABSTRACT: Silver Nanoparticles (AgNPs) are employed in a wide range of application in this study. We improved the (AgNPs) manufacturing method using marine sponge Spongiatestamethanol extract. The biosynthesis of silver nano particles were characterized based on the observations of UV – visible spectroscopy transmission electron microscopy, Field emission scanning electron microscopy analysis revealed that the synthesized silver Nano particles (AgNPs) were spherical face-center- cubic TEM (Transmission Electron Microscopy) FT-IR (Fourier Transform – Infra Red Spectroscopy and Zeta Potential. Hence, this green chemistry approach towards the synthesize of silver Nanoparticles has many application such as this process can be mold up economic viability etc.

Key words: Marine source, Spongiatosta**,** Silver Nano particles(AgNPs), SEM, TEM.

I. INTRODUCTION:

Natural products constitute the best potential library with maximum possible diversity of chemical structural types. With the advent of modern tools they have been eminently screened. The chemical complexity of the natural products thought to be a disadvantage for drug discovery is now considered as an advantage for the drug development. The oceans are full of living organisms and contain more flora and fauna compared to the land.

In the course of evolution, marine organisms have adapted excellently to the marine environment, such as high salt concentration, low temperature, high pressure and low nutrient availability. These extreme conditions require unique adaptation strategies leading to the development of new natural products, which differ from known structures of terrestrial organisms **[9]**. Drug discovery and development from the ocean was initiated about 50 years ago with the discovery of the sponge derived nucleosides spongothymidine and spongouidine [3]**.**Since then over 14,000 different natural products from marine organisms have been described [17] and hundreds of patents describing new bioactive marine natural products with several different type of biological activity such as antimicrobial, anti-inflammatory, antimalarial, antioxidant, anti HIV and anticancer activity have been isolated from marine sources. Approximately 10-15 different marine natural products are currently in clinical trials mostly in the areas of cancer, pain or inflammatory disease[6] .

Nanotechnology is appearing as a rapidly growing field with its application in science and Technology. Nanobiotechnology has emerged duo to mixing both of biotechnology and nanotechnology for developing biosynthetic and eco-friendly technology for synthesis of nanomaterial[7].Nanoscale materials display novel properties different from their bulk counterparts. The change in chemical, physical, optical, electronic and magnetic properties considerably depends on the particle size and shape [8]. The metallic nanoparticles are prepared from metal like Au, Pt, Pd, and Ag synthesized by different procedure include of physical, chemical and biological methods [9]. Physically and chemically mediated synthesis require high pressure, energy, temperature, high cost and harmfulness [10]. Use of chemical and physical procedure in the synthesis of silver nanoparticles is costly and cumbersome [11]. Investigators have used biological extracts for the synthesis of nanoparticles, by adopting simple protocols, involving in the process of reduction of metal ions by using biological extracts as a source of reduction either extracellularly [12].

Among all, nanoparticles with the unique properties in chemistry, optics, electronics, and

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magnetics have led to an increasing interest in their synthesis[13]. The most widely used and known applications of silver nanoparticles include topical ointments and creams containing silver to prevent infection of burns and wounds. Physical and chemical methods may also successfully get the pure nanoparticles but these methods are costly and potentially dangerous to the surroundings. The use of biological organisms such as microorganisms, plant extract could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner[14].

The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immuno-pharmacology. Cancer is an abnormal type of tissue growth in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cell[15]. Recently, silver nanoparticles are emerging as promising agents for drug delivery cancer therapy. The anticancer actions of nano-sized silver particles have been evaluated against a variety of human cancer cells[16].

II. MATERIALS AND METHODS:

creening and Characterization of **Marine spongeSpongiatosta**by synthesis of silver nanoparticles.

2.1Collection of sample

The sponge sample was collected as entangled specimens from a bottom trawl fish net operated off Manoli and here Islands of Mandapam group of Islands, Gulf of Mannar at Rameshwaram. It was collected by bicatching method. The samples were placed inside sterile ethyl polythene bags under water and transferred to the lab aseptically in the boxes.

2.2. Preparation of sponge extracts

Prior to the extraction, samples were washed with water, cleaned air dired, lyophilized and powdered. They were stored for further use. For the extraction of crude bioactiver, 100 g of powdered material was exhaustively extracted with 200 ml of methanol using soxhelt apparatus and concentrated in a rotary evaporated at reduced pressure.

2.3. SYNTHEIS OF SILVER NANO PARTICLES AND SPECTRAL STUDIES

Silver nitrate was purchased from Sigma Chemicals Company, MO, USA. All the other chemicals were purchased from Hi-Media Laboratories Pvt. Ltd **(**Mumbai, India**)**.

2.3.1. Fourier Transform-Infra Red Spectroscopy (FT-IR)

This is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures and has been used as requisite method to and identify medicines in pharmacopoeias of many countries. FT-IR a quick and effective analysis method used for the complicated mixture system had played an important role in pharmaceutical analysis in recent years [29]. Due to the inherent complexity of IR spectrum, the actual interpretation may be difficult and the operation requires much experience. Indeed slight differences in the spectra within the same plant species may not be obvious and generally not visible to the naked eye. Thus, the application of IR Spectroscopy in herbal analysis is still very limited compared to its application in other areas **(**Food and beverage, Pharmaceutical**)**.[17]

2.3.2. Bio synthesis of silver nanoparticles

10 ml fresh and dry sponge methanolic extract was mixed with 100 $AgNO_3$ solution 1mM in a 250 ml Erlenmeyer flask. The whole mixture was placed in to a stirrer at 70°C **(**700 rpm**)** for 4 hour and kept in the dark. The bio reduction of $AgNO_3$ ions achieved within 4 hour continuous stirring. A color change to yellowish. The solution were centrifuged at 12000 rpm for 30 min in 4°C and then the pellets were suspended in deionized water and again centrifuged at 8000 rpm for 15 min. The purified silver nanoparticles were freeze dried and stored at 4°C for further use The extract attained was followed by Millipore filter **(**0.45 µm**)** and used at 4°C for additional experiments. The final yield of Spongiatosta is 12.5 Gv/v.

2.4. Characterization of silver nanoparticles

After the incubation period, the silver nitrate treated methanolic extract of AgNPs-Marine sponge Spogiatosta was centrifuged at 9,000 rpm for 15 min. The supernatant was taken for the analysis of size, shape and stability of the bioreduced silver nanoparticles using Scanning Electron Microscope **(**SEM**)**, Transmission Electron Microscope **(**TEM**)** and Fourier Transform Infra Red Spectroscopy **(**FT-IR**)**.

2.4.1. UV-Vis Analysis

The optical property of AgNPs was determined by UV-Vis spectrophotometer **(**Perkin-

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Elmer,Lamda 35,Germany**)**. After 3the addition of $AgNO_3$ to the sponge extract, the spectras were taken in different time intervals up to 24Hrs. between 350 nm to 500 nm. Then the spectra was taken after 24Hrs. of $AgNO_3$ addition.

2.4.2. SEM analysis of silver nanoparticles

Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. The excess solution was removed using a blotting paper. The film on the SEM grid was dried under a mercury lamp for 5 min. The thin film on grid was examined using Scanning Electron Microscope.

2.4.3. TEM analysis of silver nanoparticles

Samples for TEM analysis were prepared by placing a drop of the silver colloidal solution on a TEM copper grid **(**200 meshes, carbon-coated, colloid on covered**)**. The films on the TEM grids were dried and the excess solution was removed using blotting paper. TEM measurements were performed on TECNAI 10 Philips; the instrument wasoperated at an accelerating voltage of 80 KV. The size and shape of the bio-reduced silver nanoparticles were obtained from TEM images.

2.4.4. FTIR analysis of silver nanoparticles

The $AgNO_3$ treated methanolic extract of MSST was centrifuged at 9,000 rpm for 25 min. The pellet was washed thrice with 20 ml of deionized water to get rid of free proteins/enzymes. The residue was dried and mixed with potassium bromide **(**KBr**)**. The pellet was used for FTIR analysis in the range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1}.

2.4.5. DLS & Zeta-Potential Analysis

Dynamic light scattering **(**DLS**)** which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of silver nanoparticles. The prepared sample was dispersed in deionised water followed by ultrasonication. Then solution was filtered and centrifuged for 15 min. at 25^{o} C with 5000 rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyzer**(**ZETA sizer Nanoseries, Malvern instrument Nano Zs**)**.

III. RESULT AND DISCUSSION:

3.1. Bio synthesis of silver nanoparticles

3.1.2. Visual Observation

Silver nanoparticles have applications in spectrally selective coating for solar energy absorption, optimal receptors in intercalation material for electrical batteries, polarizing filters, catalysts in chemical reaction, bio-labeling and as antimicrobial agents [18]. There are several physical and chemical methods for synthesis of metallic nanoparticles [19]. However, biological methods may be relatively simple, reliable, eco-friendly and promising [20]. Formation of silver nanoparticles by reduction of silver nitrate during exposure to methanoli extract of Marine spongeSpongiatosta extract can be easily monitored from the change in colour of the reaction mixture. The change in colour of the reaction mixture after 24 hr at 45°C is presented in Plate 2.2. The colourless solution changed into brown colour which indicates the formation of silver nanoparticles. In case of control **(**silver nitrate solution alone**)**, no change in color was observed. The colour change was due to excitation of surface Plasmon vibrations in the metal nanoparticles. This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range. This observation was reconfirmed by UV-Visible spectrum and XRD analysis.

The color transformation of AgNPs-MSSTmethanolextract treated silver nitrate might be due to vibrations in surface Plasmon of silver. The strong broad peak located at 430 nm indicates the reduction of Ag+ ions which further confirmed the formation of silver nanoparticles. It is corroborated to the findings of Vilchis-Nestor et al. who have reported that the noble metal silver displays characteristic absorbance at around 430 nm. It has been suggested thatbioactive components are mainly responsible for the reduction of silver ions. Thus, the study suggest that active constituents contents of MSSTextract might reduce Ag+ into Ag^{0}.

Plate 3.1. Marine spongeSpongiatosta

Plate 3.2. Colour change in reaction mixture **(**silver nitrate +MSST**)**

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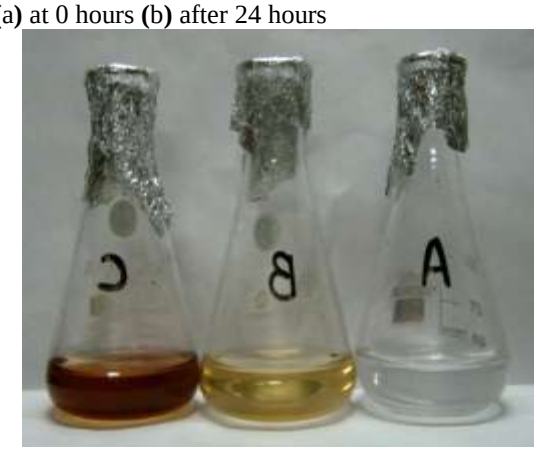
(a**)** at 0 hours **(**b**)** after 24 hours



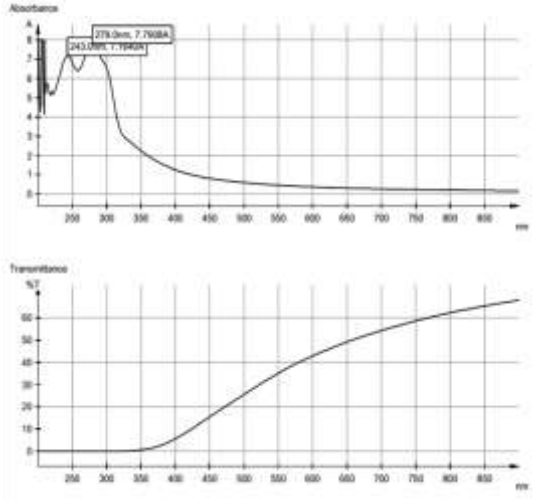
A: Silver nitrate solution
B: Silver nitrate + MSST
C: Silver nitrate + MSST **(**After 1 hr**)**

3.1.3.UV-Vis PECTROPHOTOMETER ANALYSIS

Reduction of silver ions into silver nanoparticles during exposure to MSST extracts was observed as a result of the color change. The color change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles were observed around 279 and 243 nm in case of MSST(Figure 2.12).

The intensity of absorption peak increases with increasing time period. This characteristic color variation is due to the excitation of the SPR in the metal nanoparticles the insets to, represent the plots of absorbance at **λmax(**i.e., at 279nm**)** versus time of reaction. The reduction of the metal ions occurs fairly rapidly; more than 90% of reduction of Ag+ ions is complete within 4 Hrs. after addition of the metal ions to the MSST extract. The metal particles were observed to be stable in solution even 4 weeks after their synthesis. By stability, we mean that there was no observable variation in the optical properties of the nanoparticle solutions with time. The formation and stability of the reduced silver nanoparticles in the colloidzal solution was monitored by a UV-Vis spectrophotometer [21].

Figure 3.3. Graphical representation of UV-Visible spectra of AgNPs synthesized from methanolic extract of MSST



3.1.4. Scanning Electron Microscope (SEM)

A further insight into the surface morphology, size and shape of the synthesized AgNPs was provided by SEM analysis. This plate 2.3. shows the SEM image of AgNPs synthesized from methanol extract of MSST of AgNPs synthesized from methanol extract of MSST. The particles are predominantly spherical in shape. The availability of biomolecules in the extract has resulted in the synthesis of spherical AgNPs.The size of the particle is reduced to micro and nanoparticles which passes through sieve no: 88 [22] so that the drug is easily assimilable in the digestive system. The scanning electron microscope study revealed the cluster arrangement which is evident from the micrograph, may be due to the presence of various chemicals, fibrous materials in the herbal sample and the presence of nano and micro sized particles. The size and surface of nano and microparticles are easily controlled to attain drug targeting in both active and passive ways[23]. Nanoparticles extend window period of bioavailability and defend the drug from enzymatic and chemical disintegration**,**[24] result in reduced peripheral side effects of drugs. The nano and micro particles together present in the drug resulting in a better bioavailability and facilitates absorption. A further insight into the surface morphology, size and shape of the synthesized AgNPs was provided by SEM analysis. This plate 2.3 shows the SEM image of AgNPs synthesized from methanol extract of MSST. The particles are predominantly spherical in shape. The availability of biomolecules in the extract has resulted in the synthesis of spherical

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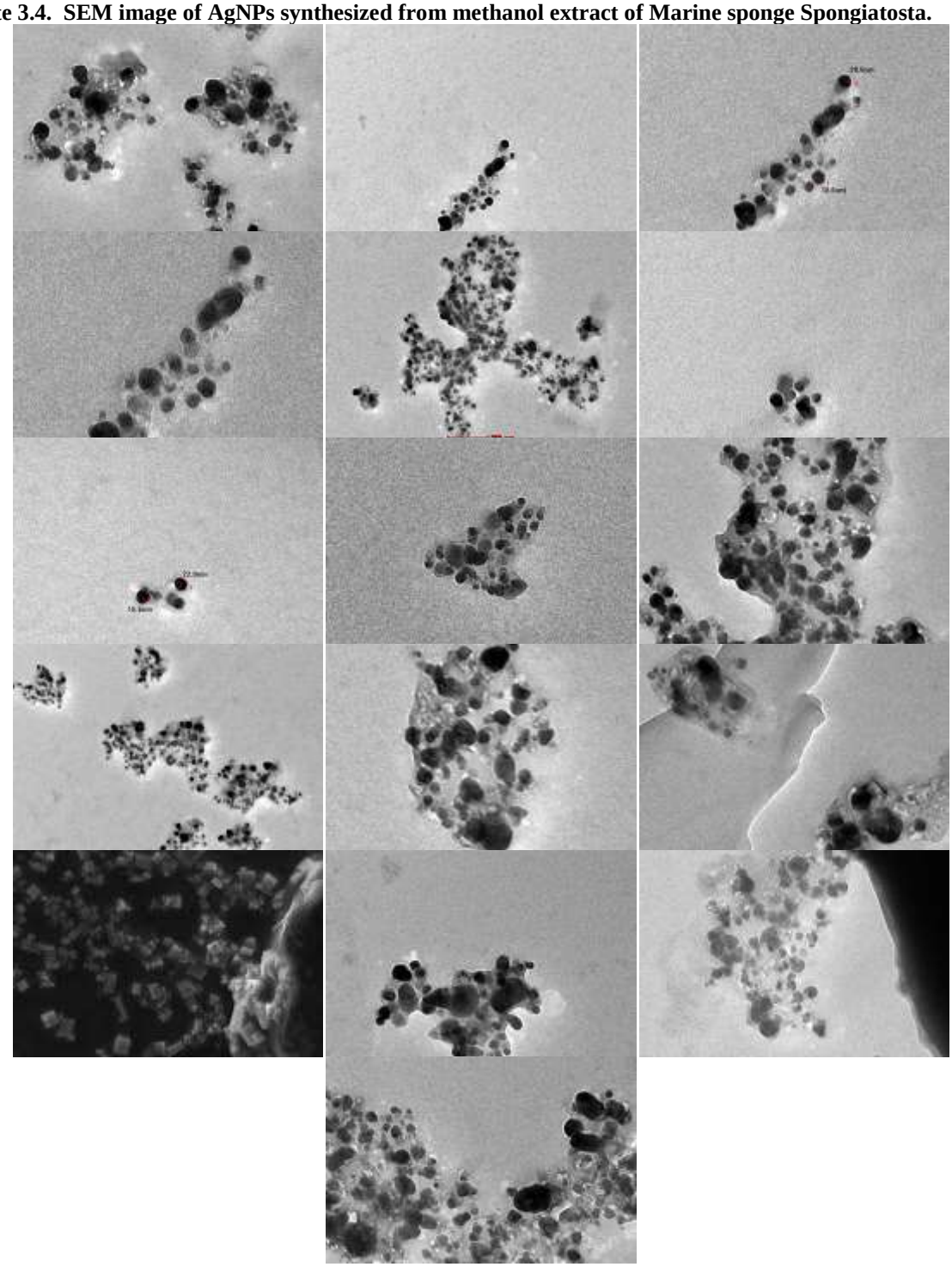
AgNPs.

Plate 3.4. SEM image of AgNPs synthesized from methanol extract of Marine sponge Spongiatosta.



1.5.PARTICLE SIZE ANALYSIS

The particle size determination of the formulated nanoparticles was shown based on intensity. Particles size analysis shows that, whwn scanning from 1nm, the particles count is very low and its gradually reached the higher value at 76 nm and again it gradually decreased.So this indicates that the maximum nanoparticles in the range of 50

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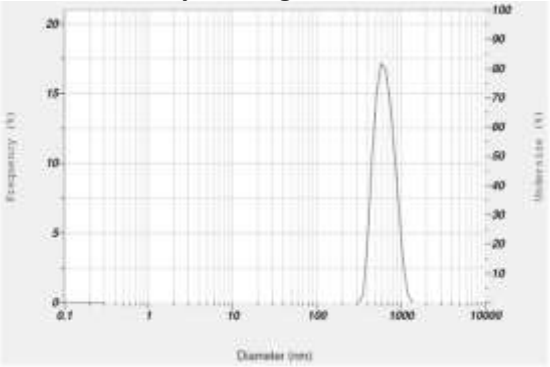
to 76 nm and only very few particles are present below and above this range Figure 2.13 particles are present below and above.

Figure 3.5. Graphical representation of Particle Size analysis of AgNPs–MEMSST



3.1.6.Transmission electron microscope (TEM)

Transmission electron microscopy experiment proved the formation of silver nanoparticles, shown in plate 2.4 Most of the silver nanoparticles were spherical in nature and often agglomerated into small aggregates, comprising of 4-5 particles each (Plate 2.4). The obtained nanoparticles were quite uniform in size and ranging between 14-26 nm.

The plate 3.6. showed the HR-TEM analysis

which revealed the synthesized AgNPs were found to be mono-dispersive in nature with the shape homogeneity. The average size distribution was found to be in rage of 14-26 nm in diameter and spherical in shape. A transmission electron microscope was employed to analyze the size, shape and structure of the nanoparticles that were formed.

The average size distribution was found to be 16 nm in diameter and shape was predominantly spherical in shape. HR-TEM images recorded from drop coated films of the silver nanoparticle synthesized. TEM was used to study the size, shape and structure of the silver nanoparticles. It is known that spherical as well as non-spherical nanoparticles exhibits better physical properties if they are produced small in size, as the therapeutic properties of silver nanoparticles are size dependent [25].

3.1.7. EDAX Analysis

In Figure 2.14, the EDAX analysis showed strong signal in the silver region and confirmed the formation of silver nanoparticles. Metallic silver nanocrystals generally showed typical optical absorption peak approximately at 3keV due to Surface Plasmon Resonance **(**SPR**)**[26]. There is also a strong signal S for K, Mg, Fe, Ca and C in the EDAX analysis. These trace quantities of minerals quantitatively assessed by EDAX analysis along with carbon, oxygen may play an important role in the functioning of various enzymes in biological systems and have immunomodulatory functions and thus influence the susceptibility to the course and the outcome of a variety of viral infections. Magnesium is a cofactor that regulates diverse biochemical reactions in the body- including synthesis of proteins, functions of muscle, nerves, controlling blood glucose and regulation of blood pressure[27]**.**Calcium is responsible for the bone remodeling and serves as a main storage material in bone. These elements are present in minimal quantities and heavy metals like arsenic, lead, cadmium and mercury are below the detectable limit and hence strengthened the safety profile.

From a technological point of view, the obtained silver nanoparticles have potential applications in the medical field and this simple product has several advantages such as cost effectiveness, compatibility for medical and pharmaceutical applications, as well as, large-scale commercial production.

Plate 3.6. HRTEM micrograph of MSST silver nanoparticles

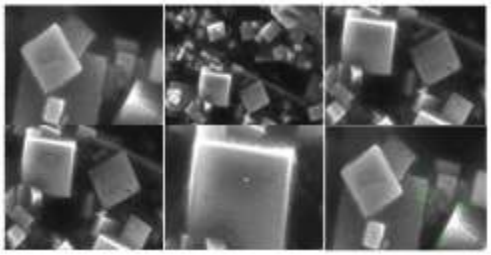
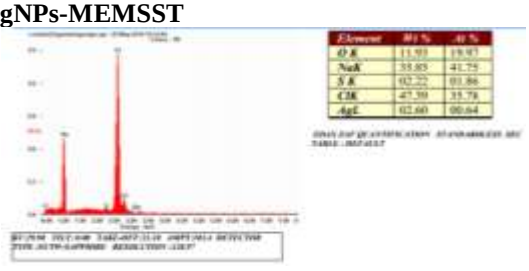


Figure.3.7. Graphical representation of EDAX AgNPs-MEMSST



3.1.8. ZETA POTENTIAL

The particle size distribution of the AgNPs was shown under different categories like size distribution by volume and by intensity. The average diameter of the particles was found to be

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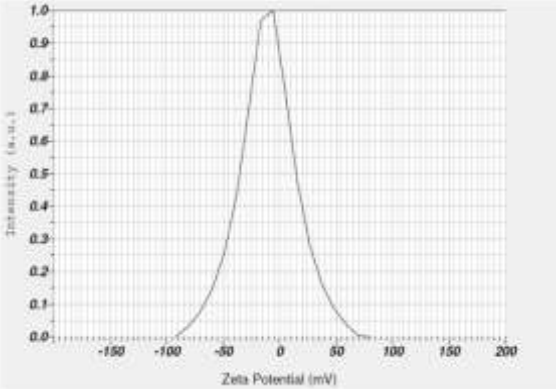
73nm **(**100% intensity**)** with a zeta potential −24mV **(**Figure 2.15**)**. The synthesized AgNPs were well distributed with respect to volume and intensity indicating well dispersed AgNPs.

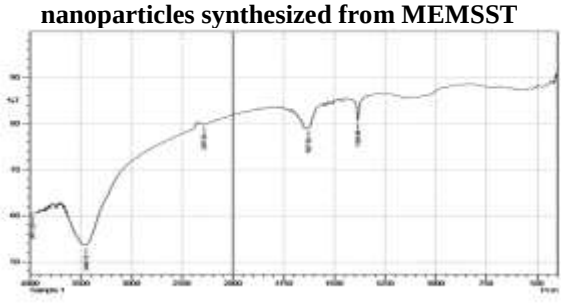
Figure 3.8. Graphical representation of Particle Size Distribution of AgNPs by Intensity with Zeta Analyzer Zeta Potential of AgNPs produced by MEMSST



3.1.9. FTIR spectroscopy analysis

FTIR analysis was used for the characterization of the methanol extract of Marine spongeSpongiatostaand the resulting silver nanoparticles. FTIR absorption spectra of water soluble extract before and after reduction of Ag^{+} ions indications the capping ligand of the silver nanoparticles may be an aromatic compound or alkanes and carboxyl group exhibited in Figure 2.16.

Figure 3.9. FT-IR Spectrum of silver nanoparticles synthesized from MEMSST



The absorbance band was observed at 1384.88 **(**cm^{-1}**)** and 1627.92 **(**cm^{-1}**)** assigned to the C-H and C=C stretch alkanes group respectively. The band at 2207.58 **(**cm^{-1}**)** corresponding to the S-H bend Mercaptans group. The band seen at3448.72 **(**cm^{-1}**)** corresponding to the O-H stretch Carboxylic acids group. The result revealed that the capping ligand of the silver nanoparticles may be an aromatic compound or alkanes. From the analysis of FTIR studies we confirmed that the carbonyl group from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles **(**i.e., capping of silver nanoparticles**)** to prevent accumulation and thereby stabilize the medium. This recommends that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium[28].

IV. CONCLUSION

The present investigation is the first report which used Marine sponge Spongiatosta for the production of silver nanoparticles. The results showed that the extract of Marine sponge Spongiatosta is capable of reducing silver nanoparticles extracellularly. This technique is simple, rapid, and no toxic method without any physical or harmful chemicals. From a technological point of view, the obtained silver nanoparticles have potential applications in the medical field and this simple product has several advantages such as cost effectiveness, compatibility for medical and pharmaceutical applications, as well as, large-scale commercial production. The eco-friendly green mediated synthesis of silver nanoparticles using MEMSST extract was attained successfully. Therefore, this green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as ease with which the process can be sealed up economic viability etc.

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