Eco- Friendly Synthesis of Silver Nanoparticles using a Sea Weed, Kappaphycus Alvarezii (Doty) Doty ex P.C.Silva

Ganesan. V*, Aruna Devi. J, Astalakshmi. A, Nima. P., Thangaraja. A

Abstract - The present study is aimed to investigate the ability of Kappaphycus alverazii to reduce silver nitrate into silver nanoparticles. The sea weed, Kappaphycus alverazii was collected from the coasts of Rameswaram, Ramanathapuram district of Tamil Nadu, India. The sea weed broth was prepared and resuspended in an aqueous solution of 1mM silver nitrate in 250ml Erlenmeyer flask. This mixture is known as reaction medium. The reduction of silver nitrate into silver nanoparticles in the reaction medium was monitored by keeping it in an incubator cum shaker with 250 rpm at 27°C for 96hours. From this reaction medium, a small aliquot of the sample was used for the characterization of silver nanoparticles through UV-Visible spectroscopic analysis, Fourier Transform Infrared (FTIR) spectral analysis, X-Ray diffraction (XRD) analysis, Scanning Electron Microscopic (SEM) and Energy Dispersive X-ray (EDX) analyses. The time dependant spectral analysis provides the evidence of synthesis of nanoparticles. The FTIR analysis explains the stability of silver nanoparticles that are synthesized by the sea weed. The XRD analysis gives the structural information of nanoparticles. The SEM and EDX analyses confirmed the synthesis of nanoparticles. Thus eco-friendly synthesis of silver nanoparticles is achieved using the sea weed, Kappaphycus alverazii, as there is no involvement of toxic chemicals as reducing agents in this biological synthesis.

Index Terms—***Kappaphycus alverazii, silver nanoparticles, eco-friendly synthesis, sea weed broth, reaction medium.***

I. INTRODUCTION

Nano-science is currently a fast growing niche and Nanotechnology is at the cutting edge of this rapidly evolving area [1]. Nanotechnology collectively describes technology and science involving nano scale particles that increases the scope of investigating and regulating the interplay at cell level between synthetic material and biological system [2]. It can be employed as an efficient tool to explore the finest process in biological processes [3] and Biomedical Sciences [4]. One of the most important criteria of Nanotechnology is the development of clean, non toxic and environmentally acceptable "Green Chemistry" production, involving organisms ranging from microbes to higher plants [5]. However, the term "Green Nanotechnology" has emerged with a lot of attention and includes a wide range of processes that reduce or eliminate toxic substances to the environment [6]. The nano scale plays an important role in manipulating biological processes that will focus biomedical and biological issues which need an approach of either Nano science or Nanotechnology [7]. Besides this, nano particles play an indispensable role in drug delivery, artificially implants and tissue engineering. The developmant of this technology in nano-medicine is playing the way for the possibility to diagnose the diseases. In the recent past, the nanoparticles of the noble metals like silver, gold and platinum have been synthesized using a variety of methods including hard-template [8], bio-reduction [9, 10 and 11] and the use of microbial cells [12]. In addition, the biological synthesis of metal nanoparticles using higher plants is currently under exploitation [13, 14]. Among the noble metal nanoparticles, silver nano particles play a significant role in the field of Biology and medicine. As the silver nanoparticles have large surface area relative to their volume, they interact with other particles and increase their antibacterial efficiency [15]. Though such silver nano particles can be produced by physical and chemical methods, they provoke problems of environmental concerns. The naturally available biological resources can be alternative sources for the biosynthesis of nanoparticles [16,17,18]. The sea weeds are rich in biologically active substances that may reduce the silver nitrate and hence biosynthesis of nanoparticles using sea weeds has turned much attention towards the utilization of renewable marine resources [19]. However few reports are there regarding the usage of sea weeds in the green synthesis of silver nanoparticles [20, 21, 22, 23, 24, 25, 26]. The present study is therefore aimed to design a protocol for alternative eco-friendly synthesis of silver nanoparticles using sea weed, *Kappaphycus alverazii*, as there is involvement of neither toxic chemicals nor reducing agents.

II MATERIAL AND METHODS

The fresh and healthy thallus of the sea weed, *Kappaphycus alvarezii* (Doty) Doty *ex* P.C.Silva. (Family: *Solieriaceae*) was collected from the coasts of Rameswaram, Ramanathapuram district, Tamil Nadu. *Kappaphycus alvarezii* (Syn. *K. cottonii*) is a species of red alga. This alga grows to two meter long and is green or yellow in color. The collected sea weed was thoroughly washed with tap water for one hour followed by double distilled water and then air dried at room temperature for one week.

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The dried sea weed (5g) was cut into small pieces and boiled in 50ml distilled water for 5-10 minutes in a water bath and the temperature was adjusted to 90°C. The extract was filtered through double layered cheese cloth. This filtered solution is known as sea weed broth.

10ml of the freshly prepared sea weed broth was resuspended in 190ml of aqueous solution of 1mM silver nitrate in 250ml. Erlenmeyer flask [14, 27]. This mixture is known as reaction medium. The reduction of silver nitrate to silver ions in the reaction medium was monitored by keeping it in an Incubator cum shaker (ORBITEK) with 250 rpm at 27° C for 96 hours. From this reaction medium, a small aliquot of the sample was used to characterize the silver nano particles synthesized during the above reaction. The UV-visible spectroscopic analysis has been performed by using a UV-1650CP Shimadzu spectrophotometer which was operated at a resolution of 1nm as a function of reaction time. The XRD analysis was performed by using a PANALYTICAL – XPERT PRO MPD (Multi – purpose powder X-ray diffractometer) with nickel filtered CuKά radiation (40KV, 300mA) of wave length (λ = 1.54060). SEM analysis was performed by just dropping a very small amount of the sample on a metal grid coated with carbon film and dried it gradually at room temperature. This sample was then sputter coated with gold and visualized with a JOEL SEM with 50,000X magnification and coupled to an EDAX-DX-4 Energy dispersive X-ray system operated at 120kV.

III RESULTS AND DISCUSSION

A. UV-Visible spectra of silver nanoparticles

UV-Visible absorption spectroscopy is one of the main techniques to examine the size and shape of the nanoparticles in aqueous suspensions [28, 29]. The result of the present study divulges that the reduction of the silver nitrate leads to the formation of silver nanoparticles and completes in 96 hours of reaction. The milky white solution at zero hour reaction (Fig.1A) changed into grey after 24 and 48 hours reaction (Fig.1 B and C) and finally turned in to black in colour after 96 hours of reaction (Fig.1D) which indicates the formation of silver nanoparticles from the silver ions. The Surface Plasmon Resonance (SPR) is highly influenced by shape and size of the nanoparticles [30]. The SPR of the grey and black coloured solutions of the present study shows the vibration maxima as a fuction of reaction time.

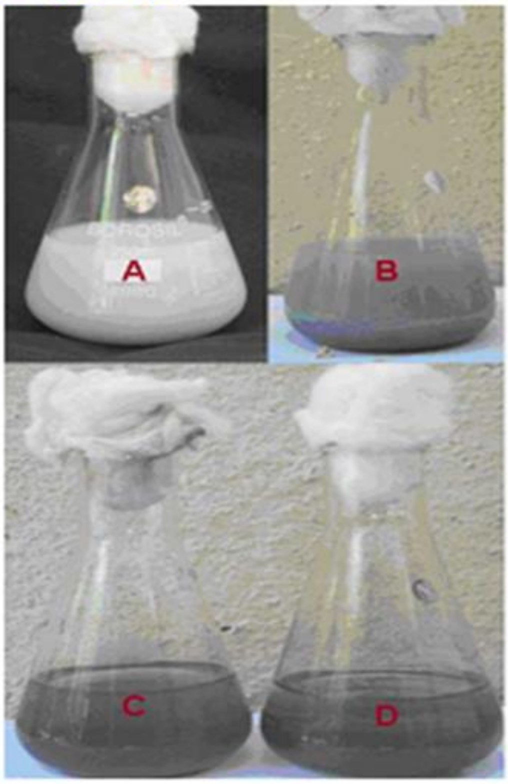

Fig.1 Erlenmeyer flasks showing the sea weed broth of *Kappaphycus alvarezii* with silver nitrate solution started colour change from milky white to black. A) At Zero hour, B) After 24 hours, C) After 48 hours and D) After 96 hours

UV-Visible spectra show no evidence of absorption in the range of 300-600nm for the sea weed broth alone but shows a distinct absorption maximum at around 420nm synthesized by silver nanoparticles using sea weed broth (Fig 2). The surface plasmon resonance (SPR) of silver nanoparticles varied with the substrates or an organism by which they are synthesized. The peaks of silver nanoparticles synthesized by *Fusarium semitectum* is 443nm [31], *Klebsiella pneumoniae* is 430nm [32] and higher plants is 430nm [33, 34]. However, the shoulder at 370nm corresponded to the transverse plasmon vibration in silver nano particles whereas the peak at 440nm due to the excitation of longitudinal plasmon vibrations [35]. In the present study the surface plasmon peak of silver nanoparticles synthesized by the sea weed *Kappaphycus alvarezii* is 420nm (Fig.2). The maximum absorption occurs at 420nm and steadily increases in intensity as a function of reaction time. The final absorbance intensities at 420nm increases up to 0.4a.u. The unchanged surface plasmon vibrations which were recorded as a function of reaction time clearly indicate the stability of newly formed silver nanoparticles .This stability of silver nanoparticles was assessed using FTIR spectroscopic analysis.

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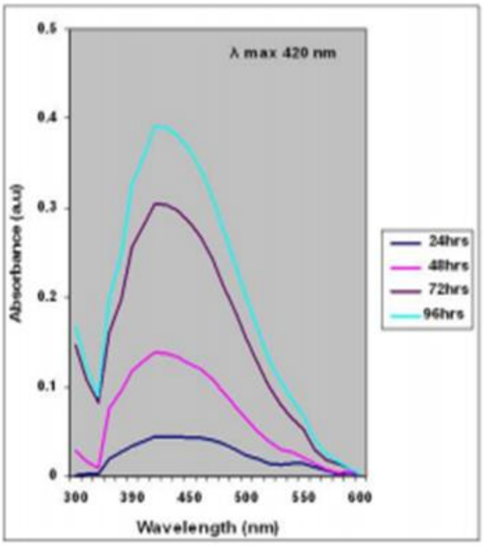

Fig.2 UV-Visible absorption spectra recorded as a function of time of reaction at 1Mm aqueous solution of silver nitrate with sea weed (*Kappaphycus* alvarezii) broth

B. FTIR spectroscopic analysis

FTIR spectroscopic analysis was carried out to identify the possible biomolecules and cell metal ions interaction in the sea weed broth responsible for the stabilization of the newly synthesized silver nanoparticles [36, 37]. The FTIR spectrum of the stabilized silver nanoparticles synthesized using sea weed broth (Fig. 3) shows the peaks that are observed at 601.75, 1106.1, 1123.46, 1136.96, 1191.93, 1400.22, 1672.17 and 2879.52 cm^{-1} respectively. This peak might be contributed by the C-O groups of the polysaccharides in the biomass of seaweed broth. Further it is suggested that the asymmetric $-CH_3$-bending modes of methyl groups of protein [38] may be responsible for the reduction of silver nitrate ions.

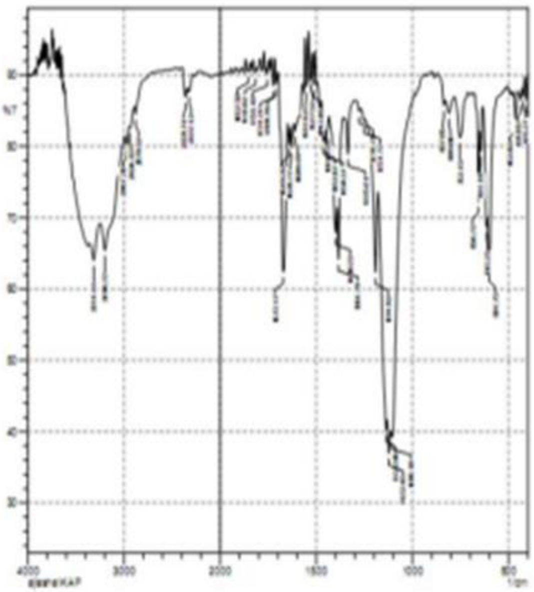

Fig.3 FTIR Spectrum of Silver Nanoparticles synthesized by exposure of aque silver nitrate to sea weed (*Kappaphycus alvarezii*) broth

The peaks at 1106, 1123, 1136 and 1191 cm^{-1} correspond to Polysaccharides [39] and C-O stretch associated with Glycogen [40]. The absorption peak at 1400 cm^{-1} is due to C-N, deformation N-H and deformation of C-H [41]. The peak at 1672 cm^{-1} corresponds to C=C stretching of alkenes [42]. The band at 2879 cm^{-1} is characteristics of C-H stretching and symmetric structure of methoxy groups present [43]. FTIR analysis of the present study clearly reveals that polysaccharides and -C-O groups of glycogen present in the biomass of the sea weed broth may be responsible for the reduction of silver nitrate in to silver nanoparticles. Further, the synthesized silver nano particles were analyzed through XRD analysis to get structural information of the nanoparticles.

C. XRD analysis

The X-ray diffraction patterns obtained for the silver nanoparticles synthesized using sea weed broth is shown in (Fig. 4). The presence of remarkable intense peaks at 2Ө values of 46 from 116 and 32 form 150 form the lattice plane of face centered cubic silver unequivocally indicates that the particles are made of pure silver [44]. According to Scherer's formula [45], t = 0.91/B cos Ө, an average size (t) of the silver nanoparticles can be estimated from the X-ray wavelength of the CuKα radiation (1= 1.54 Aº), the Bragg's angle (Ө), and the width of the peak at half height (B) in radians. The average size of the silver nanoparticle synthesized by exposure of aqueous silver nitrate with *Kappaphycus alvarezii* broth is 74nm. Further SEM and EDX analyses were performed to strengthen the structural information synthesized silver nanoparticles. XRD analysis substantiated that the silver nanoparticles had been synthesised through bioreduction of silver ions to silver nanoparticles by the sea weed.

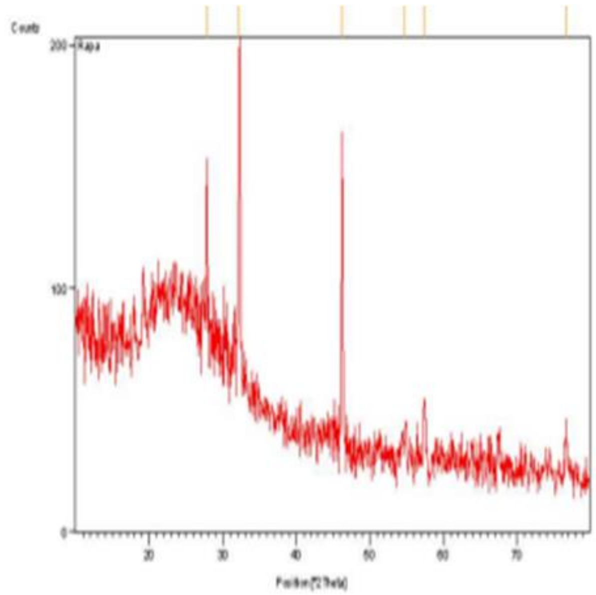

Fig.4 XRD Spectrum of Silver Nanoparticles synthesized by exposure of aqueous silver nitrate to sea weed (*Kappaphycus alvarezii*) broth

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D. SEM and EDX analyses

Scanning Electron Microscopic analysis (SEM) has proved further surface morphology and size details of the silver nanoparticles. A representative SEM image recorded from the silver nanoparticles at 40000 X (Magnification) is shown in (Fig. 5). The silver nanoparticles were found to be spherical in structure with an average size is around 73 nm.

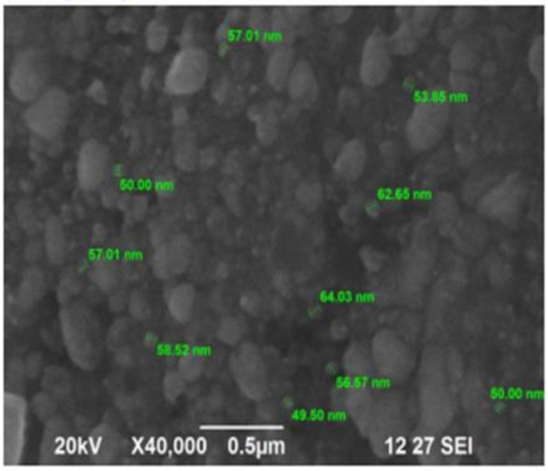

Fig.5 SEM micrograph of silver nanoparticles synthesized using the reaction of aqueous silver nitrate with sea weed (*Kappaphycus alvarezii*) broth at 40,000X magnification

EDX spectra of silver nanoparticles are shown in (Fig.6). EDX analysis confirmed the significant presence of elemental silver. EDX profile has shown a strong silver signal (73.67%) along with a weak signals of oxygen, calcium, magnesium, chlorine, silicon and aluminium, which may originate from the biomolecules that are bound to the surface of the silver nanoparticles [46]. Thus the silver nanoparticles were synthesized through the bioreduction of silver nitrate using the seaweed, *Kappaphycus alvarezii*. In the present study it is believed that the aminoacids present in the biomass of sea weed may be the reducing agent which reduces the silver ions to silver nanoparticles.

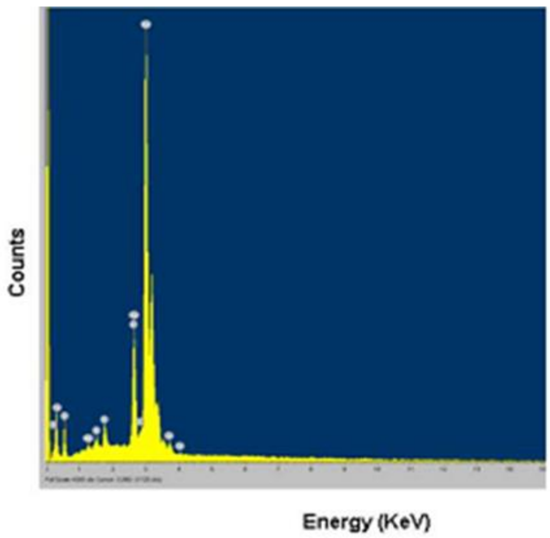

Fig.6 EDX spectra of silver nanoparticles synthesized using aqueous silver nitrate with sea weed (*Kappaphycus alvarezii*) broth

IV CONCLUSIONS

Thus the present study explains an eco-friendly approach using sea weed, *Kappaphycus alvarezii* in the synthesis of silver nanoparticles at room temperature. Only the period of 96 hours was required for the complete reduction of silver ions to silver nanoparticles and the average particle size could be 74nm. The nanoparticles with different sizes obtained from the reaction medium can directly put forward to various biomedical applications because of the green technology procedure in their synthesis This is the route in which there is no involvement of any toxic or hazardous reducing agents, capping or dispersing agent. Thus the eco-friendly synthesis of silver nanoparticles has been achieved in the present study using sea weed, *Kappaphycus alvarezii*. It also can be scaled up for large scale synthesis of silver nanoparticles without using either high pressure, energy, costly chemicals as reducing agents

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