WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 01, 1322-1330. Research Article ISSN 2277– 7105

BIOSYNTHESIS OF SELENIUM NANOPARTICLES USING CITRUS RETICULATA PEEL EXTRACT

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Article Received on
06 Nov 2014,

Revised on 29 Nov 2014,
Accepted on 21 Dec 2014

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ABSTRACT

In the past 60 years, antibiotics have been critical in the fight against infectious diseases caused by bacteria and other microbes. Due to increased resistance against antibiotics developed by the microbes, there has been a steep decline in their usage worldwide. Selenium is an essential trace element with a narrow margin between beneficial and toxic effects. Selenium has effect on microbes which are essential to clinical applications. An environmentally friendly route has been used for synthesizing selenium nanoparticles using an orange peel extract as both reducing and stabilizing agent. The characterization of the nanoparticle synthesis were conducted at various temperatures ranging from 20˚C to 80˚C and pH ranging from 2 to 12.The orange peel extract was found to be more efficient in reducing sodium selenite to selenium nanoparticle of spherical shape. The synthesized nanoparticle were characterized using ultraviolet (UV)-Vis spectrophotometer and scanning electron microscopy. The anti-algal activity of the selenium nanoparticles were tested and found to be effective in inhibiting algal blooms.

KEYWORDS: Sodium Selenite, nanoparticles and Scanning Electron Microscopy.

INTRODUCTION

Biological synthesis of nanoparticles by waste source of plant extracts is at present under exploitation as some researchers worked on it [1][2] and testing for antimicrobial activities. [3][4][5] For the last two decades, extensive work has been done to develop new drugs from natural products because of the resistance of micro-organisms to the existing drugs. Nature has been an important source of a products currently being used in medical practice.[6]

Synthesis of nanoparticles by green methods with antibacterial properties is of great researcher's concern in the explored of new pharmaceutical and biomedical products.

The synthesized selenium nanoparticles are greatly enhanced because of tiny size. Nanoparticles have immense surface area relative to volume. Therefore, minuscule amounts of selenium nano particle, can lend antimicrobial effects to hundreds of square meters of its host material. Nanomaterials are the leading requirement of the rapidly developing field of nanomedicine, and bionanotechnology. Selenium has effect on microbes which are essential to clinical applications. According to previous study, metal nanoparticles are very good antioxidant and less toxic than other forms. [7]

Algae form an integral part of the environment. The algae in study have been isolated from tannery water and the algae were cultured in BG11 medium. The algae form a major problem in tannery industries by adhering to the tank surfaces. This inversely affects the BOD and COD levels of water discharged into the lakes and rivers. The ultimate effects of algal growth are eutrophication, leading to aquatic death. Tannery water being a source of ferrous, potassium and ammonium salts [8] favors the growth of the algae. The study to evaluate the effect of Selenium nanoparticles on algal growth was conducted and analyzed.[12] The increase in death was verified using various methods and confirmed. The possible mechanism for the algal death due to selenium nanoparticles has been referred and verified. The biochemical pathway followed by the selenium nanoparticles in the algal cell is also illustrated.

The orange peel extract has an enzyme which reduces sodium selenite to selenium nanoparticles, which is synthesized at different temperatures and pH. The presence of selenium ions, neutralized sample was scanned in UV- Vis spectrometer (Perkin Elmer Lambda 35) using quartz cuvettes from 200-700nm.This endowed us to adopt the technology for biosynthesis of nano particle specially emphasizing on selenite nanoparticles.

MATERIALS AND METHODS

Preparation of the extract

Orange (*Citrus reticulata*) was bought from the local markets of Sathyamangalam. Exactly about 50 g (±0.005) of the orange peel were weighed and were stored in sterile conditions. The weighed peels were then ground in a mortar and pestle until a fine mixture having sticky and wetted appearance is got. The mixture is then boiled in 150 ml of double distilled water

for 15 mins until the color of the water turns orange. It is then filtered using Whatmann Filter paper no.1. The extract is used as necessitated in the following characterization experiments.

Effect of temperature: 50 ml of the extract is taken on a temperature controlled magnetic stirrer at varying temperatures of 30ºC, 40ºC, 50ºC, 60ºC, 70ºC and 80ºC. 5 ml of sodium selenite (Na_2SeO_3) is added a concentration of 0.1M in drops until the formation of selenium nanoparticles is confirmed by the formation of red color. The presence of selenium nanoparticles were characterized by UV-spectroscopy at 275nm.

Effect of pH: 50 ml of the same extract was considered for the biosynthesis of nanoparticles by varying the pH parameter as 2, 4, 6, 8, 10 and 12. The pH is optimized using pH meter and the pH was adjusted using acidic solution containing 10% HCl and for base, 1N sodium hydroxide was utilized. 5 ml of sodium selenite (Na_2SeO_3) is added a concentration of 0.1M in drops until the formation of selenium nanoparticles is confirmed by the formation of red color. The presence of selenium nanoparticles were characterized by UV-spectroscopy at 275nm.

Collection and Cultivation of Algae

Algal cells were collected from the tannery water and were subjected to washing using running distilled water to remove unwanted epiphytes and associated debris. The cultivation of the algal cells was done in BG11 medium and tannery water was supplemented to it in the ratio 10:1. This was done to increase the growth rate using tannery nutrients. The culture was maintained at 25±1ºC with an illumination of 14.8 dyne.cm^2 from a white fluorescent tube light for 19 hours per day.

Anti-algal assay

The total algae grown in BG11 were separated and from it, 1 gram was again separated from it. From the separated algal cells, 0.5 grams of the algae (S_0) was subjected to chlorophyll extraction using acetone as solvent. A standard graph on chlorophyll content of various concentrations of algae was derived from 6 samples with varying concentrations of algae from 0 to 1 gram. Varying concentrations of algae were inoculated in 100 ml of BG11 with 10 ml supplement of tannery water and labeled S_0 to S_6. The algae were subjected to 15 ml of the sample containing SeNPs each. The algal cells (S_0-S_6) were then maintained at 35ºC for 48 hours under 24 hour illumination.

RESULTS AND DISCUSSION

Characterization of Orange Peel Extract: Orange peel represents a complex storehouse of myriad of biomolecules like ascorbic acid, vitamin A etc. The extract was characterized by the decreased pH (5.2) of the solution due to the presence of citric acid in the extract. The other properties of the extract revealed that it was faint orange in color.

Characterization of Se NPs: The selenium nanoparticles synthesized at varying conditions were confirmed by a full scan module of the sample in a UV- Vis spectrophotometer (Perkin Elmer Lambda 35) using quartz cuvettes from 200-700nm. A peak was observed at 265.5 nm (Figure 1) confirming the presence of selenium in the samples. The size and structural morphology of the nanoparticles were later established by using scanning electron microscope studies and it was confirmed to be 70nm in size, further corroborating the evidence (Figure 2).

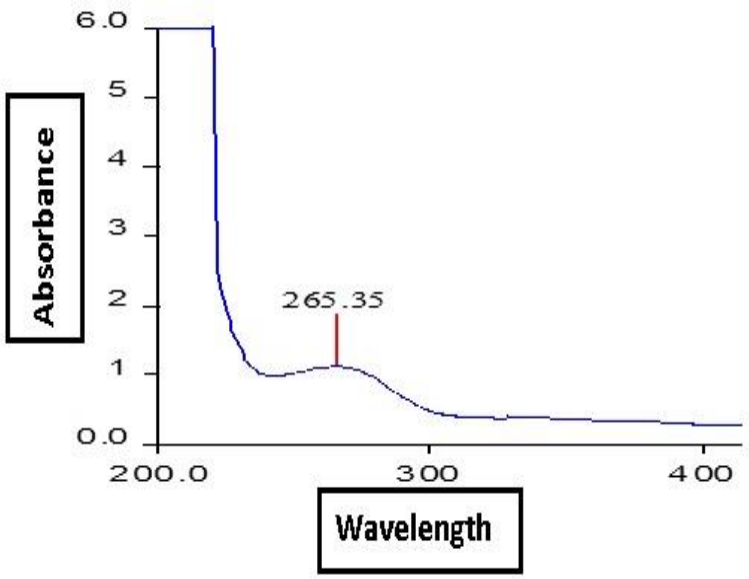

Figure 1: figure shows the absorbance maximum between 200 to 400nm. SeNPs shows an absorbance maximum at 265nm.

Effect of temperature: The time taken for conversion of sodium selenite to selenium nanoparticles was considered the reduction activity point in all the tests. Time required was found to be indirectly related to the reduction capability of the extract. It was found that the conversion of selenium salt to its nanoparticles was found to be efficient at 40ºC (Figure 3). A steep decrease in the reduction capacity of the orange peel extract was observed when the temperature was increased exponentially.

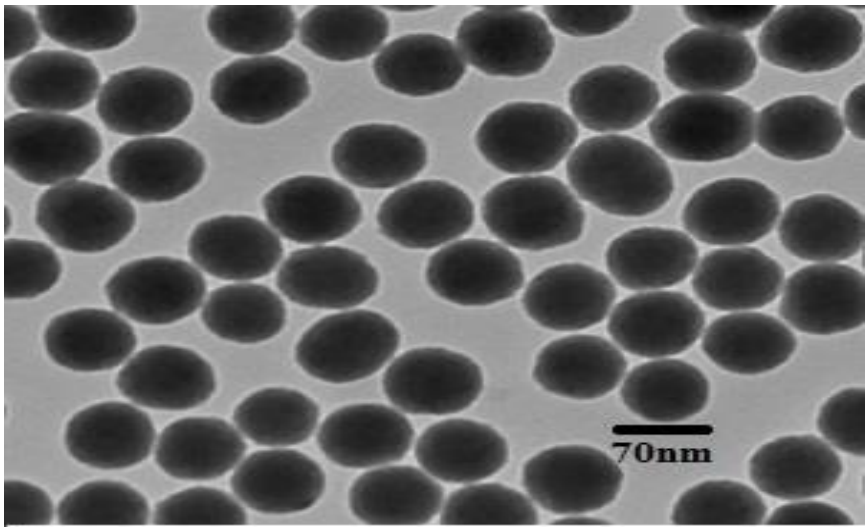

Figure 2: Scanning Electron Microscopy image of Selenium nanospheres.

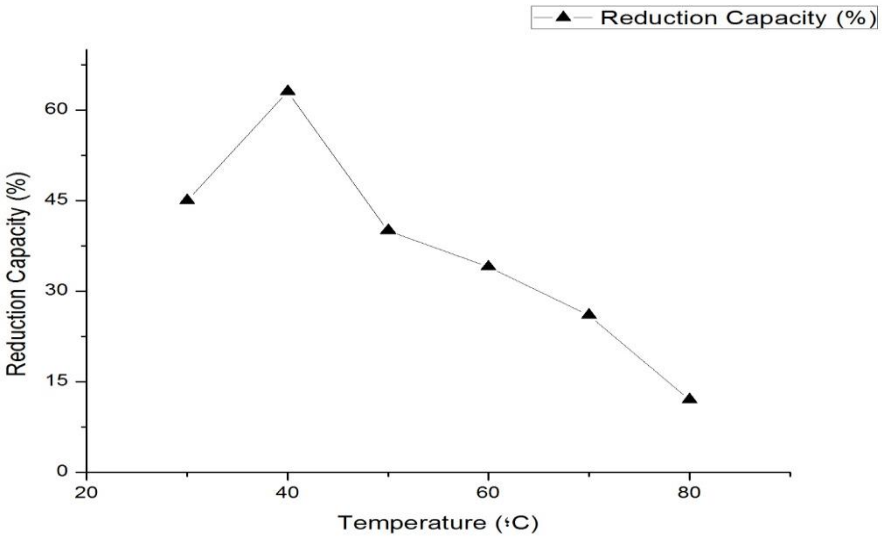

Figure 3: Effect of temperature on reduction capability of extract

Effect of pH

The reduction activity for this variable was also determined as a measure of time. The time interval was calculated at various pH and it was found that the selenium salts were exceptionally reduced when the pH of the extract was maintained at 4 (Figure 4). The reduction was hindered only to a negligible value when the pH was increased and showed the minimum towards the alkaline portion of the scale.

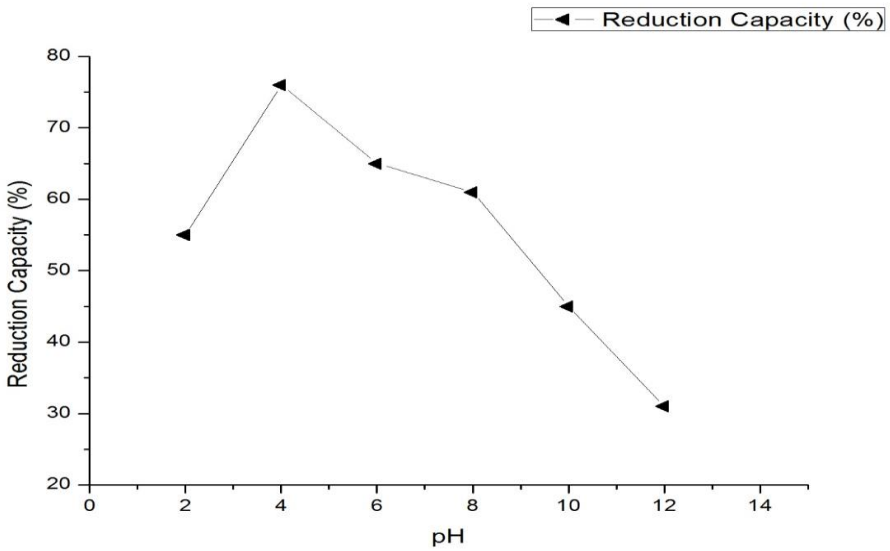

Figure 4: Effect of pH on reduction capability of extract

Table 1: Table showing variation in the concentration of the viable algal cells with respect to time after treatment with SeNPs.

Sample	Time(hr)	Absorbance(OD)	Concentration(W/V)%
S_0	0	0.205	1.0835
S_1	1	0.157	0.8360
S_2	5	0.135	0.7226
S_3	10	0.0956	0.5195
S_4	15	0.0754	0.4154
S_5	20	0.0564	0.3175
S_6	24	0.0421	0.2438

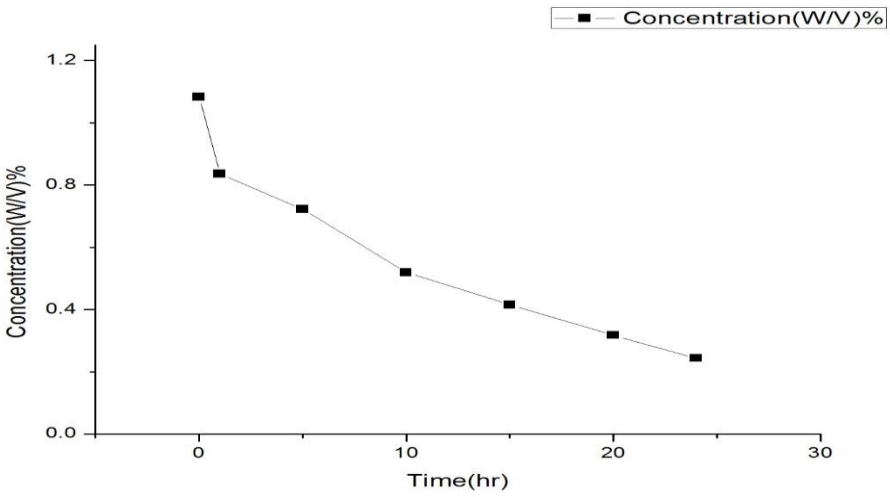

Figure 5: Graphical analysis of variation in the chlorophyll concentration with respect to time. A linear decrease is observed until the 24th hour.

Anti-algal assay: The algal cells were visually confirmed for death and the chlorophyll was extracted using acetone as solvent. The absorbance values for the samples were taken at 625nm.[11] [12] The acetone extracts of S_0 showed an absorbance of 0.205 and that of S_6 showed 0.0421(Table 1). A graphical analysis of the absorbance and the time showed a linear decrease in the chlorophyll content with respect to time (Figure 5). The decrease in the chlorophyll content ultimately represents the number of viable algal cells in the culture. The difference in OD value thus confirms the decline in algal growth due to SeNPs. When the concentration of Se increases, it forms SeO_4^{2-} and the pathway leads to the formation of DMSe which potentially becomes toxic to the algae in large amounts and since the surface to volume ratio of the algal cells is high, it accumulates the DMSe in large amounts ultimately causing death. The conversion of SeNPs in an organic system to selinate has been studied before [7] (Figure 6).Thus, the application of nano selenium particles for the death of algal cells is found viable. The toxicity of the nano Se particles is also less effective and Se can be recovered back by carbon disulphide precipitation [9] or by reverse osmosis, thus leaving the threat of selenium nanotoxicity covered. The pathway has to be further analyzed for dose dependent studies of SeNPs.

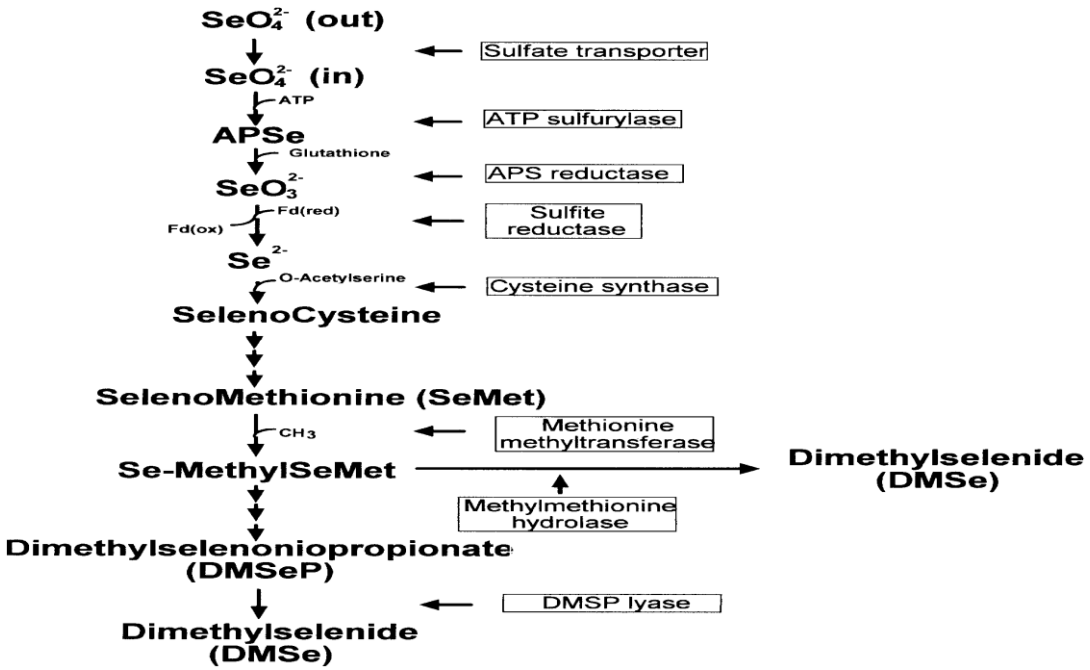

Figure 6: The biochemical pathway for the production of DMSe is illustrated above [10]

ACKNOWLEDGEMENT

We thank The Chairman, Bannari Amman Institute of Technology, Sathyamangalam for helping us through the research.

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