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Biogenic Producing Selenium Nanoparticles by *Clostridium Perfringens*

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ABSTRACT

Since past ages up to now there is different bacterial and microbial infection which through food, staff and other ways could cause different untreatable in human and animals. In these ways there are different antibacterial ways for threating of the sickness but in the modern world at the same time with development of different science such as nanotechnology, different study have been done on produce and feature of antibacterial effect of nanoparticles such as silver and gold. In continue of this study different ways for producing one reliable and less expensive nanoparticles in progressing which consequently using of the nanomaterials in different medical, food and industrial science could give a bright perspective for using exact and less expensive treatment against different bacterial and microbial has opened of modern man. One of the best ways of production nanoparticles is utilizing biologic methods by the microorganism themselves. Such ways are not only less expensive but also more economic and purer and they cause that side effect in human binges decreases.

In present study production of nanoparticles of selenium which has been produced in biogenic way by bacteria of *Clostridium Perfringens* has been studied for the first time. This bacterium can cause of clinical illness such as Gangrene, but this bacterium can produce nanoparticles of selenium in natural environment in storing and intracellular forms.

In this study production of biometric nanoparticles by *Clostridium Perfringens* with great stability has been studied and size and quality of nanoparticles in comparison with nanoparticles of selenium produced of *Klebsiella pneumonia* have been evaluated. Findings show that selenium nanoparticles produced by *Clostridium Perfringens* have more purity and stability in comparison with selenium nanoparticles produced by *Klebsiella pneumonia*.

Nanoparticles of selenium which has been produced in comparison whit other methods such as biogenic with amount, purity, and stability which by using this bacterium there is possibility for nanoparticles to by extracted through spreading nanoparticles to extracellular environment will be possible.

KEYWORDS: nanoparticles, *Clostridium Perfringens*, biogenic, Nano, selenium

INTRODUCTION

Today nanotechnology could have great influence on different sciences. This could create context with antibacterial new situation. According to this study it has been recognized that Nano selenium has different feature such as antioxidant. With considering the past, we can understand the materials such as selenium oxide and selenium sulfate since very past ages have been used as antifungal in threating of fungi illness. [1, 3, 5]

In production of metallic nanoparticle there are two general ways i.e. up and down and down and up. But each of these wayshas different production methods [2, 4]. In methods of physics and by using metal grounding by very advanced tried to grind metallic picas as size as Nano metric that this method is too expensive and very less purity.[6] In physics and chemistry some methods such as chemical recovery, utilizing ultra sound waves, and the mass of steam and the bio production methods are and methods of the production down to up and in this method there was an attempt to put the atoms and molecules of metal for producing of nanoparticles according necessary sizes and providing physics chemistry methods, interaction of materials and chemical solution on production environment can again produce nanoparticles whit less purity.[8.9] Chemical materials and solution which has been used can cause some great damages and toxicating of the researcher but in different bio method these nanoparticle by using of one celled and multi celled organisms which can get base on down to up method.[7] In this context the using of the bacteria have got so much attention. For example in this context we can mention to gold nanoparticles which have been produced by some bacillus and fungus such as aspergilluses. [10]

According to this subject producing nanoparticles based on physics chemistry methods has both hi expense and disadvantages such as misbalancing and lack of stability in size of nanoparticles, and lack of controlling the growth of nanoparticles. So in conclusion, producing of nanoparticles with bio methods as a clean method of producing has been considered intensely.[11]

In continuing of the study this point is very important that producing selenium nanoparticles with biologic method is not new and by using some microorganisms this production has been done but in this study with considering the goal of producing selenium nanoparticles with less purity and small size has been tried to use

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Clostridium Perfringens bacterium and finally optimized its production by *Klebsiella pneumonia* has been studied.[12]

MATERIAL AND METHOD

Providing species and bacterial culture

Bacteria *Clostridium Perfringens* and *Klebsiella pneumonia* have been provided from factory of Razi vaccine and serum production in south east of Iran. The bacteria have been taken by sterilized loop and transformed to culture of TSB which already had temperature of 121 c for 20 minutes by using sterilized laboratory autoclave and allowed to growth anaerobic bacteria condition to OD600=1after that this inoculums have been used for produce nanoparticle of selenium by *Clostridium Perfringens* and *Klebsiella pneumoniae*

Preparation of Se nanoparticles

Se nanoparticles were made according to the recipe described in the literature. Briefly, a 200-mg/ml ion of selenium equivalent (564-mg of selenium oxide) was mixed with a 100-mL fresh tiptic soy broth media (TSB) that sterilized at (121oC, 17 psi, 15 min) and supplemented with 1% (v/v) of *Klebsiella pneumonia* and *Clostridium Perfringens* inoculums, system incubated at 37℃ for 24 hours. Periodically, aliquots of the reaction solution were removed and the absorption was measured in a UV-Vis spectrophotometer (Seilent diode array) at 200-300 nm. *Klebsiella pneumonia* and *Clostridium Perfringens* cells containing red selenium particles were disrupted using a wet heat sterilization process in a laboratory autoclave at 121°C, 1.2 kg/cm2 for 20 minutes. The released selenium nanoparticles were centrifuged at 25000 g for 15 minutes and washed three times with distilled water. The washed sample was confiscated for 10 minutes (Tecna6, Techno-Gaze, Italy).The physical properties of selenium nanoparticles were characterized by Transmission Electron Microscopy (TEM) and its chemical properties were confirmed by Energy Dispersive Spectroscopy (EDS), respectively.

RESULTS AND DISCUSSION

This study investigated biogenic produce of selenium nanoparticles by *Clostridium Perfringens* and *Klebsiella pneumoniae*. Both of these bacteria could produce nanoparticles of selenium. The prepared aqueous solution of Se nanoparticles produced by both of these bacteria shows an absorption band at 210 nm as shown in Figure 1, which is a typical absorption and of spherical Se nanoparticles due to their surface Plasmon. [13, 15]

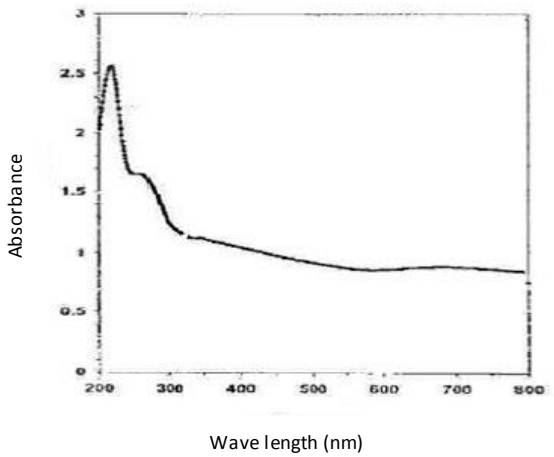

Fig.1: UV-vis spectrum recorded as a function of time of reaction in a solution of 200 mg/ml with the *Clostridium Perfringens* and *Kelebsiella pneumonia*

Shape and size distribution of the synthesized Se nanoparticles were characterized by transmission electron microscopic (TEM)study. The biogenic selenium nanoparticles released after the sterilization process and three time washing with distilled water and a few drops of Se nanoparticle solution were dropped onto a TEM grid, and the residue was removed by a filter paper beneath the TEMgrid. The TEM image shown in (Figure 2) was obtained by high-resolution TEM (JEOL, JEM-2000E7).

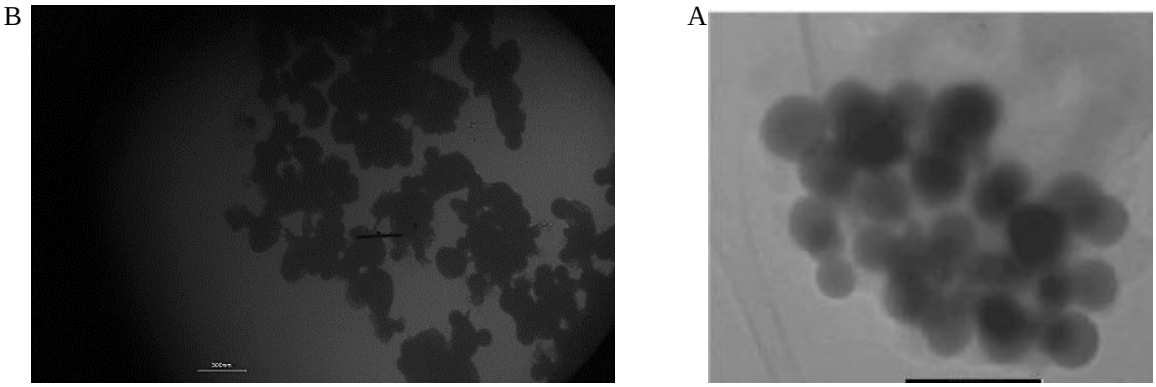

Fig.2:A): TEM image of nanoparticles dispersed on a TEM copper grid for *Clostridium Perfringens*. B): *Klebsiella pneumoniae*

As can be seen by the shape and size distribution in that figure, the particles are highly monodispersed and histogram of selenium nanoparticles (figure3), indicates the existence of produced nanoparticles in the ranges of 43 to 531 nm with an average size of 198 ± 85 nm for *Klebsiella pneumonia* and 28 to 300 nm with an average size of 120 ± 50 nm for *Clostridium Perfringens*.

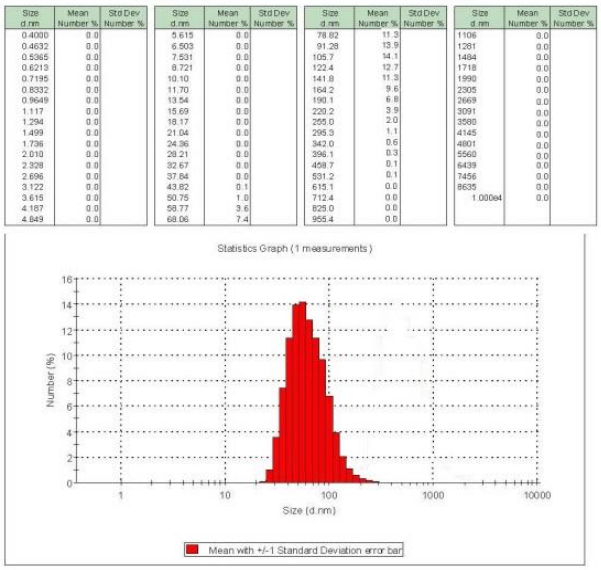

Fig3: A histogram showing size distribution of Se nanoparticles produced by *Clostridium Perfringens*

CONCLUSION

This study demonstrated the possibility of using biological synthesized selenium nanoparticles and their incorporation in materials, providing them sterile properties. [16]

In the presence of both *Klebsiella pneumonia* and *Clostridium Perfringens* in anaerobic conditions Selenium ions in the medium, have been decreased and production of red brick were in medium, While no color change was observed in control samples. Because of the nano-particles to create new features color in environment so produce red brick on the medium, is a clear indication of produce selenium in nanoscale particles.

Stability of the nanoparticles to bind to functional groups on the surface of bacteria finds resonance and nanoparticles have been produced in this study had excellent stability after 4 weeks, However, due to differences in the functional groups on the surface of bacteria tested, manufactured nanoparticles are stable after two months for each of the bacteria used were different and this stability for nanoparticles produced by *Clostridium Perfringens* were greater than nanoparticles produced by *Klebsiella pneumonia*. Optical properties, electrical and magnetic nanoparticles, is function of the shape and size them, that control this shape and size, is associate with environmental factors. So different enzymes of bacteria used in this study, show production of nanoparticles with different sizes and shapes and stability.

The materials incorporated with these selenium nanoparticles can exhibited antibacterial activity against some microorganisms. [17,18] Although a number of different soluble metal ions or metal complexes show significant antimicrobial activity against a wide range of microorganisms, use of these materials as topical or

systematic applications have been limited for various reasons, such as toxicity to biological systems. In the case of selenium, its antioxidant and pro-oxidant effects, or its bioavailability and toxicity, depend on its chemical form. Elemental selenium powder with a redox state of 0 is not soluble in water and is generally considered to be biologically inert.[19,20]Thus, the toxicity of elemental selenium (Se0) is less than that of selenite or selenite ions. However, elemental selenium, when supplied in the form of nanoparticles, clearly can serve as a potent ingredient for the preparation of new antibacterial formulations. This is the first study to examine the produce of selenium nanoparticles by *Clostridium Perfringens*.

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