

The effect and toxicity of silver nanoparticles biology essay

[Science](#), [Biology](#)



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Introduction

This report is based on the analysis of the effect that silver nanoparticles may have on sea urchins along with references and comparisons with previous experiments involving silver nanoparticles and other marine life forms. Silver nanoparticles are used extensively in consumer products and therefore make them an ideal nanometal to perform toxicology related experiments, to determine the potential impacts they may have on the marine environment. The report itself is based on the original seminar given by Dr. Lidjia Siller of the University of Newcastle -upon-Tyne, called; " From Sea Urchin to a new inorganic catalyst for CO₂ Hydration reactions". The seminar gave an overview of the: Mineralization ProcessesStorage of CO₂Bio mineralization and its connection to Sea UrchinsExperimentation by exposing the Sea urchins with Silver Nanoparticles. Analysis methods such as TEM [EDX and EELS], STXM (scanning transmission X-ray microscopy) & FTIR (Fourier Transform Infra-Red microspectroscopy). This report will focus on the effects of silver nanoparticles on sea urchins as well as comparisons with several other experimental conditions involving silver nanoparticle intoxication with other subjects and how the particles may enter the marine ecosystem in the first place. However, the prime aspect of this report is the agglomeration of the silver nanoparticles within the sea urchins.

Silver, Silver Nanoparticles and Sea Urchins

Silver itself has been used in a wide variety of applications such as jewelry, silverware, electronic components, photography, etc. However in addition to

that it has also been used extensively in the medicinal industry due to the discovery of its antifungal and bacterial properties. Hence why soluble silver salts have been used in treating mental illness, epilepsy, nicotine addiction, gastroenteritis, and infectious diseases, including syphilis. This is why the study of the toxicology of silver is a necessary study, as the number of uses in daily life has grown considerably and the possible ways of the residual silver nanoparticles to enter the environment have grown. Thus the study of the effects on marine ecology is necessary as the increased amount of silver deposition within the earth's waters might have large consequences on the ecological system. Sea urchins were the chosen specimen for the toxicology study, specifically the *Paracentrotus lividus* or more commonly known as the purple sea urchin, because of the role they play within the marine environment. Being part of many such food webs means that the sea urchins population is critical to the balance of marine stability, hence making it a prime candidate to determine how the silver nanoparticles may affect marine life on a large scale. Their grazing limits algal biomass and they live in environments that alternate between two stable states: luxuriant, species-rich kelp forests and sea urchin-dominated "barrens." They are a candidate in toxicology studies for several reasons: They spawn a large number of gametes which can be easily obtained and externally fertilized. Fertilization can be properly manipulated and carried out in laboratory conditions. Embryo development can be studied within a few days. During early development stage the embryos are very sensitive to pollutants and different kinds of stresses. They provide a suitable model organism for both

ecological/developmental studies and biomineralization processes. This makes sea urchins a cost effective and efficient subject for the research.

Experimental Method

Silver Nanoparticle Preparation Method:

The silver nanoparticles were synthesized by the chemical reduction of silver compounds using the following precursors: Silver Nitrate [AgNO₃] - 0.

0043g De-ionized water - 95mL; 18 MΩ-cm Sodium citrate [Na₃C₆H₅O₇] - 5

mL The procedure for the synthesis of the silver nanoparticles is as follows;

Silver nitrate solution is dissolved in the deionized water. Once dissolved the solution is heated by a hot plate to boiling point. Once at boiling point

sodium citrate in calculated amounts is added to the solution. The resulting

solution is then refluxed (To boil (a liquid) in a vessel attached to a

condenser so that the vapors continuously condense for reboiling.) for about

30 minutes, so that the solution is turned into a silver nanoparticle

suspension. After the suspension the temperature was decreased to room

temperature, it was stored in the dark at 4-8 °C before use. The silver

Nanoparticle sizes were determined using a transmission electron

microscope.

Sea urchin sample Preparation:

The sea urchin samples require special preparation techniques as this will involve creating embryos in laboratory conditions and ensuring that they are

suitable for the experiment. For the control sea urchin sample preparation,

the steps are as follows: Sperm and eggs (the gametes) are obtained from

adult sea urchins. Fertilization of the gametes is induced using an injection of

0. 5M KCl. This is carried out in natural sea water, to replicate normal fertilization conditions. For the exposed sea urchin sample preparation, the steps are as follows: In the case of the silver nanoparticle exposed samples, about 2 hours after fertilization, the silver nanoparticles are added to the sea urchin larvae, at a concentration of 0.3 mg/L. In both cases, the larvae are collected after 51 hours by fixation with ethanol, and they are washed several times with De-Ionized water before any measurements are made.

Experimental Results

The analysis methods of this experiment include: X-ray Absorption Near Edged Spectroscopy (XANES) Fourier Transform Infra-red microscopy (FTIR)

X-Ray Absorption near Edged Spectroscopy

X-Ray absorption near edged spectroscopy (XANES) is basically defined as the analysis of spectra obtained from X-ray fluorescence maps or X-ray absorption spectroscopy. This analysis method is specific towards elements and allows the determination of the partial density of the empty states of a molecule. The absorption edge is the key to understanding XANES; X-rays have the ability to excite a core electron of an atom to an empty one and different core electrons have different distinct binding energies, and these are unique between the elements. When the X-ray is scanning, and increases in absorption occur, these correspond to the absorption of the X-ray photon by a specific type of core electrons. This is what gives the sudden vertical rise seen in XANES spectra, and hence the name the absorption edge. The name of the edges are given according to the principle quantum number of the excited electrons, this is seen in table 1. The various energies observed

in the absorption edge in the absorption spectra, reveal the identities of any corresponding absorbing elements. In this experimentation it was used in relation with other silver compounds as a reference as seen in image 2. The spectrum from the XANES was collected in fluorescence mode on specific points of the sample at a specific beam size. The measurements were made using X-rays with tunable energy at specified ranges, with dwell times and energy step values pre-determined. An X-Ray fluorescence map, Image 1. b, of the sea urchin larva was done on a silver nanoparticle exposed sample. The Black dots on the image indicate places of silver nanoparticle agglomeration within the sea urchins. The image itself is a reverse grey scale X-Ray Fluorescence map, showing the sea urchin larva 51 hours after fertilization. Image 1. a shows the XANES spectrum of a point on the X-Ray Fluorescence Map; in this case it was focused on the point indicated by the black circle, this region is coded as AgL2, 3 edges. The presence of the peak in the region of 3. 359 keV, is assigned to the 2p to 5s transitions of silver, this indicates that the black features are agglomerated silver nanoparticles. This is another function of the XANES spectrum; it allows us to identify transition energy values, and hence characterise them.

Image 1. a

Image 1. b

Image 2, shows the XANES spectra that shows the agglomerated nanoparticles in sea urchins, image 1. a, compared with other reference spectrum from sources as, AgCl, Ag Foil, AgNO₃ , Ag₂O , AgO and Ag₂S. It is clear that the XANES spectrum obtained from the sea urchins is associated

with Silver; however there is no direct relation to any of the reference spectra.

Image 2

Even though none of the reference peaks match up directly there is some resemblance between the AgNP (Silver Nanoparticle) spectrum and that of the Ag Foil, however, in the AgNP spectrum profile there is a more pronounced peak. From this data it can be concluded that there is additional chemical bonding present in the silver nanoparticles found within the sea urchin. Image 3, Shows the comparison of the XANES spectrum of the AgNP that agglomerated in the sea urchins shown in red, with the spectrum of AgNP taken from previous experimentation on Ryegrass (*Lolium multiflorum*), done by Liyan Yin (2011), the spectrum in this case is shown in black. Both of the spectra have identical lowest peaks, first peak after point with greatest positive gradient. This peak is assigned to oxidized Ag species complexed with Sulphur and Oxygen/Nitrogen ligands. Hence the agglomerated AgNP in sea urchins are likely to contain similar silver compounds as those observed from the Ryegrass experimentation.

Image 3

Fourier Transform Infrared microspectroscopy (FTIR)

This analysis technique uses infrared radiation, and as the Infrared radiation is passed through the sample, where by some of it is absorbed and the rest of the infrared radiation is transmitted through. The spectrum that is created is a representation of the molecular absorption and transmission, which is unique to the sample. The sensitivity of the system is why it is most useful

when identifying any pollutants or contaminants within a sample, hence also making it extremely useful to identify chemical compounds. However these spectrometers differ from others as they are based on the Michelson interferometer, and the actual technique depends on the interference of infrared wave, the technique is based on the path difference of two beams recombining after being separated by a series of mirrors. The recombined beam passes through the sample. The sample absorbs all the different wavelengths characteristic of its spectrum, and this subtracts specific wavelengths from the interferogram. The detector now reports variation in energy versus time for all wavelengths simultaneously. This is the analysis technique used in the experiment to determine the contaminants and change in composition of the samples under different experimental circumstances. The FTIR spectrum was obtained from the selected regions on images 4. a and 4. b; these regions are indicated by the yellow rectangles on these images. Where image 4. a shows the control sample and image 4. b shows the sample when the sea urchin larva is exposed to the silver nanoparticles. Both of the samples have received the same amount of growth time, 51 hours so as to help determine the changes caused by the AgNP. Image 4. c shows the FTIR spectrum of absorption against wavenumber with the control (top) and exposed sample (bottom), in comparison with several reference spectrum such as; Calcite indicated in Blue, Sodium sulphate [Na₂SO₄] indicated in red, and sodium thiosulphate [Na₂S₂O₃. 5H₂O] indicated in green. In the image the peak for calcite at 864cm⁻¹ is stronger in the control sample than the exposed sample. Calcite is a fundamental compound in the growth of sea urchins, brought about by

the activity of the enzyme carbonic anhydrase; so the lack of calcite within the exposed sample can be explained by the AgNP possibly affecting the activity of the enzyme, hence inhibiting the calcite formation within the sea urchin larva.

Image 4. a

Image 4. c

Image 4. d

Image 4. b

Image 4. d shows the FTIR spectra within the ranges of 900 to 1300 cm^{-1} enlarged. This is to simply the comparing of the exposed sample indicated in black, with the sodium sulphate (red) and sodium thiosulphate (green).

When comparing the spectra together, it is clear that the exposed sample contains sulphur compounds; this may be due to a biological response from the larva, in order to reduce the concentration of the silver nanoparticles that have been introduced. The results correspond to the XANES (image 3) stating that the oxidized Ag species observed are complexed with both Sulphur and Oxygen/Nitrogen ligands in AgNP-exposed sea urchins.

Conclusion

The sea urchin larva that were exposed to the silver nanoparticles at a concentration of 0.3 mg/L developed agglomerations of silver nanoparticles within them, this is seen from the image 1. b, the X-ray fluorescence map which shows the nanoparticles as black clumps, however they don't seem to agglomerate in any specific pattern across the sea urchin larva. From the

XANES result it is seemingly possible to conclude that the compounds within the silver nanoparticles might be oxidized and complexed with sulphur and oxygen/nitrogen ligands. As explained this can be seen by using reference spectra of different silver compounds and comparing it with the silver nanoparticle spectra. It is from the FTIR spectra that we can see an increase in sulphur production within the sea urchins that have been exposed; hence it can be related to an increased biological process to reduce the toxic elements within the sea urchin. These are the of the experimentation with the sea urchin embryo's and how the silver nanoparticles affects growth of the exposed sample by initiating additional reactions which increase the sulphur production within the system of the sea urchin, and also reducing the calcite production, which is a necessary compound for the growth of the sea urchin as its spines are made of calcite; therefore it can be concluded that a sea urchin embryo exposed to as much as 0.3 mg/L concentration of silver nanoparticles will undergo stunted growth.