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Synthesis, Characterization and Safety Assessment of Nano Selenium and Organic Selenium for Incorporation in Lamb Feed

S.S. Vajpeyee, J. Ramesh, R. Karunakaran, J. Muralidharan[1], V. Sankar[1]

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ABSTRACT

Background: Selenium is an important trace mineral required by the animals. It is an integral part of antioxidant system, protecting the body against free radical injury. Nano particles attract a widespread attention due to its high bioavailability and efficacy. The current study was aimed to synthesize, characterize nano selenium and evaluate the cytotoxic effect of nano selenium and organic selenium (selenocysteine) under *in vitro* condition *in vero* cell line.

Methods: Nano selenium was synthesized by wet chemical method by using sodium selenite, selenium powder, ascorbic acid and sodium hydroxide at laboratory level. In this study particle size, shape, zeta potential and selenium content were characterized by using Particle Size Analyser (PSA), Transmission Electron Microscope (TEM) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The toxicity was analysed by MTT assay against *vero* cell line.

Result: The result revealed that selenium nano particles were spherical in shape, nano in size (less than 50 nm) and pure in nature. The nano selenium and organic selenium (selenocysteine) effectively inhibited the growth of *vero* cells in a dose dependent manner.

Key words: Cell cytotoxicity, Nano selenium, Particle size, Zeta potential.

INTRODUCTION

Selenium is an essential trace element for animal health, immune function, productivity and reproductive performance in farm animals and found in both organic and inorganic forms in nature, has a specific place among the nutrients in animal feed because of its role in animal body (Mehdi and Dufrasne, 2016).

Nano mineral particles have a particle size in the range of 1-100 nm. At this scale, the physical, chemical and biological properties of materials differ fundamentally and often unexpectedly (Patil *et al.* 2012). The nano-sized particles are having higher potential than their conventional sources and would diminish the amount required (Sri Sindhura *et al.* 2014). Selenium nano-particles (Nano-Se), has novel characteristics such as high surface activity, great specific surface area, a lot of surface-active centres, strong adsorbing ability, high catalytic efficiency, high bioavailability and low toxicity (Skalickova *et al.* 2017).

Physical, chemical and biological methods can be used to produce nano selenium. The chemical method is the simplest and cost-effectivesince only a few chemicals can create nano minerals of uniform size in the laboratory (Lane *et al.* 2002). Hence, effective and controlled bulk production can be achieved by using the chemical methods. However, in chemical method there is always a chance of toxicity as hazardous chemicals are used during synthesis (Rajendran *et al.* 2013)

The chemical approach uses techniques such as chemical reduction, electrochemistry and photochemical reduction. It is reported that during synthesis process size, shape, stability and physiochemical properties of the nanoparticles are greatly influenced by variety of factors *viz* temperature, concentration, reducing agent and stabilizing agent (Alexandridis, 2011). Thus, the synthesis and characterization of selenium nano particle is utmost importance.

Each type of nanoparticle has its own distinct physicochemical features such as particle size and surface area, their toxic effects exerted on the cells may also vary. Some of the toxic effects are irreversible and permanent, leading to cell deaths and some are reversible, after the exposures to nanoparticles are removed, and the cells may begin to proliferate normally (Hillegass *et al.* 2010). The mechanism of action that leads to the cell's deaths may also be different (Hillegass *et al.* 2010). Therefore, this experiment was proposed to synthesize, characterize and

Department of Animal Nutrition, Madras Veterinary College, Tamil Nadu Veterinary and Animal Science University, Chennai-600 007, Tamil Nadu, India.
[1]Mecheri Sheep Research Station, Pottaneri-636 453 Salem, Tamilnadu, India.

Corresponding Author: J. Ramesh, Department of Animal Nutrition, Madras Veterinary College, Tamil Nadu Veterinary and Animal Science University, Chennai-600 007, Tamil Nadu, India.
Email: rameshnutrition@gmail.com

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assess cytotoxicity of nano selenium for using it as a lamb feed supplement.

MATERIALS AND METHODS

Synthesis of selenium nano particles

Nano particle of selenium was synthesized through chemical method. Nano selenium was synthesized by using two different methods and sources *viz.* sodium selenite and pure selenium powder from sigma at the Department of Animal Nutrition, Madras Veterinary College, Chennai during 2019. In the first method, nano selenium prepared by water phase solution method using selenium powder and sodium hydroxide as outlined by Kargar Razi *etal*. (2011) while in the second method, nano selenium was prepared using sodium selenite and ascorbic acid according to the modified method of Qian Le *et al.* (2010).

The yield of nano selenium was determined by weighing the product and comparing it to the precursors used. Organic selenium (selenocysteine) was purchased from sigma.

Characterization of nano selenium

When the particle size is reduced to nano size, the properties of the materials are likely to be far different from the bulk materials. In the present study, Transmission Electron Microscopy, Particle Size Analyzer, X Ray Diffractometer, Fourier Transform Infra-Red (FTIR) spectroscopy and Inductively Coupled Plasma Mass Spectrometry were used to analyze the properties like morphology, particle size distribution *etc.*

In vitro Cytotoxicity Assay

In vitro cytotoxicity assay was carried out *in vero* cell line (African green monkey kidney cell line) to ensure the safety of nano particle source of selenium as per the method of Mosmann, 1983. Serially diluted nano particles and organic selenium were incubated in ninety-six well plates containing *vero* cell line at the concentration of 10^6 cells/ml with solvent and medium following standard protocol. To this monolayer of cells in ninety-six well plates, serially diluted nano selenium was added and incubated for 24 hrs. The concentration selected were 0.25, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml. The different levels of selenium concentrations were selected based on the NRC (2007) recommendation. The samples were run in triplicates. At the end of incubation, colorimetric method measured the reduction of yellow 3- (4, 5dimethythiazol-2-yl) -2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase of live cell. The parameters like per cent cell inhibition exhibited under different concentration of nano forms of selenium and organic selenium were studied. The per cent cell activity was determined by the following formula:

Cell viability (%) = (OD of test cells/OD of control cells) × 100.

RESULTS AND DISCUSSION

The product yield (recovery %), particle size, zeta potential and selenium content in nano form of selenium are furnished in Table 1. The recovery percentage of nano particle source of selenium produced by chemical method using selenium powder as a precursor is 75.73% while yield from sodium selenite is 40.97%. The result revealed that nano selenium derived from selenium powder contain 98.34% selenium whereas nano selenium derived from sodium selenite contain 97.62% selenium.The size assayed through both Transmission Electron Microscopy and particle size analyser, confirmed that the nano particle source of selenium produced was less than 50 nm. The TEM image of produced nano selenium is presented in Fig 1 and Fig 2. Mean size assessed through Transmission Electron Microscopy is 43.46 ± 2.31 nm and 21.6 ± 2.11 nm for nano selenium derived from selenium powder and sodium selenite respectively. While mean size assessed through particle size analyser is 31.8 ± 8.90 nm and 11.97 ± 4.91 nm for nano selenium derived from selenium powder and sodium selenite respectively.

The X-Ray diffraction (XRD) pattern of synthesized nano selenium from both sources are presented in Fig 3 and Fig 4. X-Ray diffraction pattern confirms that the synthesized nano particle source of selenium was free of impurities as it does not contain any characteristic XRD peaks other than selenium peak and the samples are nano in nature. The typical FTIR spectrum of synthesized nano particle source of selenium from selenium powder showed well-defined peaks at around 3853 cm^{-1} and 1163 cm^{-1} and FTIR spectrum of synthesized nano particle source of selenium from sodium selenite showed well-defined peaks at around 3864 cm^{-1} and 969 cm^{-1}. The observed FTIR results confirmed that synthesized selenium nanoparticles were without any significant impurities. Zeta potential for nano selenium

Table 1: Product yield (recovery %), particle size, zeta potential and selenium content in nano form of selenium (Mean* ± SE).

Chemical name of source	Characterization parameters	
	Selenium Powder	Sodium selenite
Recovery (%)	75.73 ± 2.55	40.97 ± 0.79
Size (assessed through TEM) nm*	43.46 ± 2.31	21.6 ± 2.11
Size (assessed through particle size analyser) nm*	31.8 ± 8.90	11.97 ± 4.91
Zeta potential (mV)*	-33.1 ± 6.50	-37.3 ± 5.67
Selenium content (%)	98.34 ± 0.36	97.62 ± 0.14
Shape	Spherical	Spherical

*Mean of six observations.

derived from selenium powder is -33.1 ± 6.5 mV while from sodium selenite is -37.3 ± 5.67 mV assessed through particle size analyser. Nanoparticles with zeta potential values greater than +25 mV or less than -25 mV typically have high degrees of stability. Dispersions with a low zeta potential value will eventually aggregate due to Van Der Waal inter-particle attractions (Nanocomposix, 2012). Nano selenium particles produced in this study could be thus classified as having good stability.

Similar to our study Malhotra *et al.* (2016) prepared selenium nanoparticles by a wet chemical approach using ascorbic acid as a reducing agent and stabilized by coating with 10% dextrin with size of 64 ± 0.158 nm. Gangadoo *et al.* (2017) used solution phase synthesis approach for selenium nanoparticles by reducing selenium tetrachloride in the presence of ascorbic acid and recorded that nano selenium are 46 nm in size. Zhang *et al.* (2018) synthesized nano selenium with mean particle size of 36.8 ± 4.1 nm using beta lactoglobulin as a stabilizer in redox system of ascorbic acid and selenite.

The selenium content of the produced nano particle sources of selenium were same as that of the original mega particle source from which they were produced. This indicates no loss in the selenium during the synthesis process. Kargar Razi *et al.* (2011) produced the sample with 99 percent selenium content in the nano sample. Concurring with this study, nano particles of selenium having similar size and shape were produced by other researchers.

Since every crystalline material has a special pattern of diffraction, the XRD technique can be used to identify crystalline structure of nanoparticles. The broadening of the peaks in XRD confirms the formation of particles in nano size (less than 50 nm). If the nanoparticles are produced in an amorphous structure, no diffraction peak will be observed. Moreover, the smaller the nanoparticles are, the broader the XRD peaks appear (Noruzi, 2015). According to previous studies, the XRD spectrum of selenium nano particles usually have two strong and sharp reflection peaks at 2h of 24° and 30° (Cai *et al.* 2018).

The functional group of synthesized Se nano particles were analysed by Fourier Transform Infra-Red (FTIR) spectroscopy, which showed chemical bonding in a target material. Kaviya *et al.* (2011) observed the shift in the absorption band after bio reduction at 1601 cm-1 to 1584 cm^{-1} and indicated the formation of nanoparticles. The appearance of this peak was due to the presence of hydroxyl

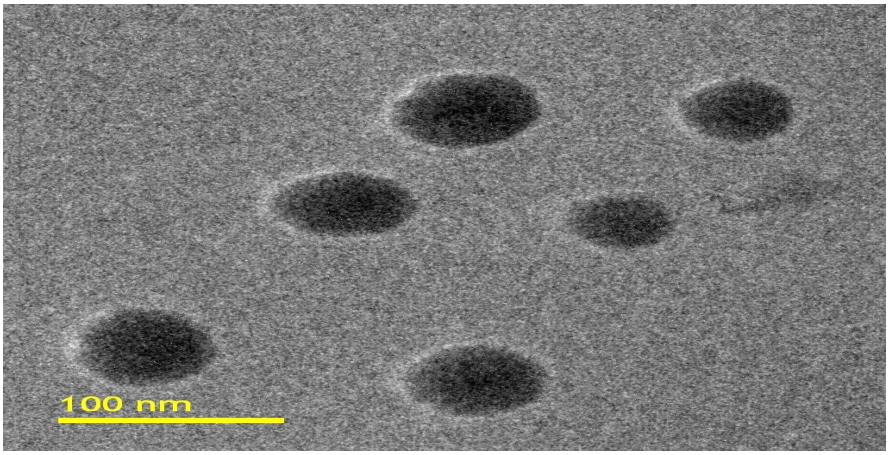

Fig 1: Transmission electron microscope image of nano selenium derived from selenium powder of size 40 to 50 nm and spherical in shape.

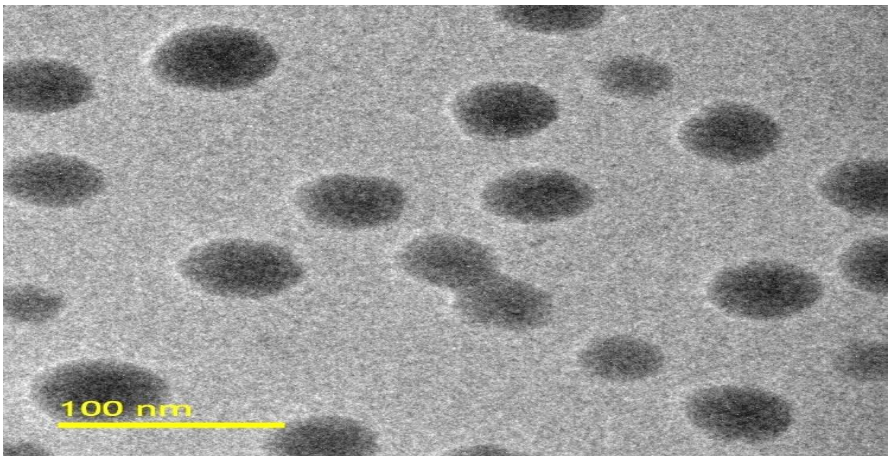

Fig 2: Transmission electron microscope image of nano selenium derived from sodium selenite of size 20 to 30 nm and spherical in shape.

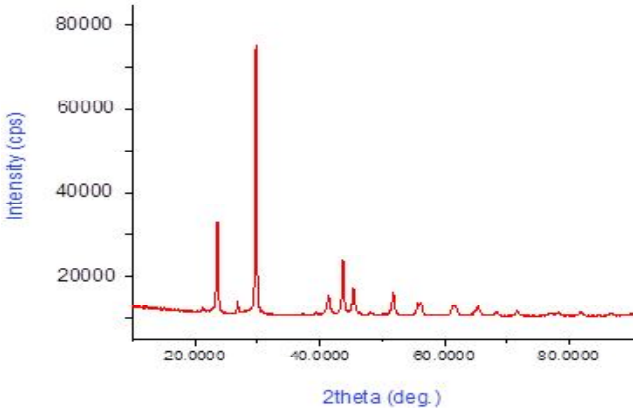

Fig 3: X ray diffraction pattern of nano selenium synthesized from selenium powder.

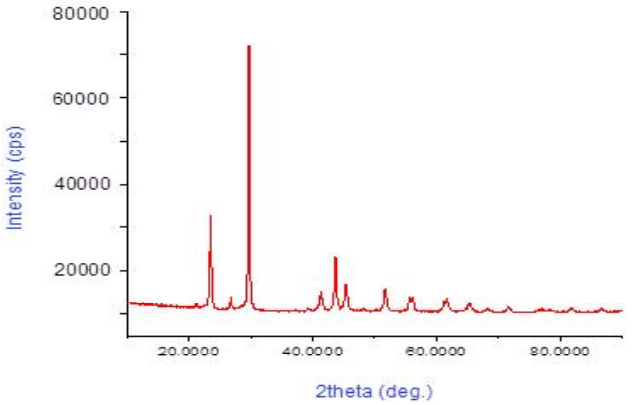

Fig 4: Xray diffraction pattern of nano selenium synthesized from sodium selenite.

group stretching vibration of phenolic compounds which was responsible for the formation and stabilization of synthesized nanoparticles.

Rudakovskaya *et al.* (2014) showed that the spherical nano particles compared to rods had higher magnetic property and stability. Thus, the nano selenium synthesised in this study possessed this advantage.

In vitro cytotoxicity study of nano selenium and organic selenium

The per cent *vero* cell death for various concentrations of organic selenium particle and nano selenium (both) is presented in Table 2 and Fig 5. The cellular activities of cells exposed to test samples were also compared with the cell control. It shows the increased concentration of nano selenium would decrease the viability of cells which is indicated by round morphology of cells.

IC_{50} (Half Maximal Inhibitory Concentration) was calculated for nano selenium derived from both sources and organic selenium. The IC_{50} is defined as the sample concentration that is required to reduce the absorbance to half that of the control and which would give the 50% cell death. Based on the calculations, IC_{50} for nano selenium derived from selenium powder was 89.11 µg/ml while nano selenium derived from sodium selenite was 85.74 µg/ml and for organic selenium it was 86.77 µg/ml. Since for nano selenium derived from both sources and organic selenium, IC_{50} value falls above 80 µg/ml, it is concluded that there is no significant difference between IC_{50} values of nano selenium derived from both sources and organic selenium.

Alam *et al.* (2019) reported that IC_{50} value of selenium nanoparticles against CHO pro-cells was obtained to be 88

Table 2: Effect of different selenium sources on percentage cell viability of African Green Monkey Kidney (*VERO*) cell line determined by MTT assay (Mean ± S.E.).

Concentration, µg/ml	Nano Selenium 1	Nano Selenium 2	Organic Selenium
	% of cell viability	% of cell viability	% of cell viability
0.25	100.00 ± 1.21	99.96 ± 0.07	99.79 ± 0.17
0.5	99.79 ± 0.34	99.87 ± 0.04	99.65 ± 0.08
1	99.58 ± 0.44	99.06 ± 1.44	98.88 ± 2.38
2	99.37 ± 0.56	98.20 ± 1.11	98.11 ± 0.22
5	99.16 ± 0.90	91.41 ± 0.32	97.48 ± 0.16
10	97.07 ± 1.44	89.69 ± 2.11	94.55 ± 0.07
20	84.91 ± 0.05	89.57 ± 0.33	91.54 ± 0.54
30	75.26 ± 2.34	78.79 ± 1.88	89.45 ± 0.66
40	69.18 ± 2.19	75.72 ± 1.34	82.88 ± 0.88
50	63.10 ± 2.11	74.78 ± 0.13	76.45 ± 1.47
60	61.63 ± 0.06	69.09 ± 1.12	72.40 ± 1.85
70	58.28 ± 1.22	62.12 ± 0.43	61.22 ± 0.71
80	55.97 ± 1.01	52.71 ± 1.13	51.43 ± 2.34
90	54.30 ± 0.99	42.84 ± 1.81	40.74 ± 1.92
100	49.69 ± 0.33	40.44 ± 1.53	38.92 ± 2.55

*Mean of three independent experiments.
Nano selenium 1 derived from sigma selenium powder.
Nano selenium 2 derived from sodium selenite.

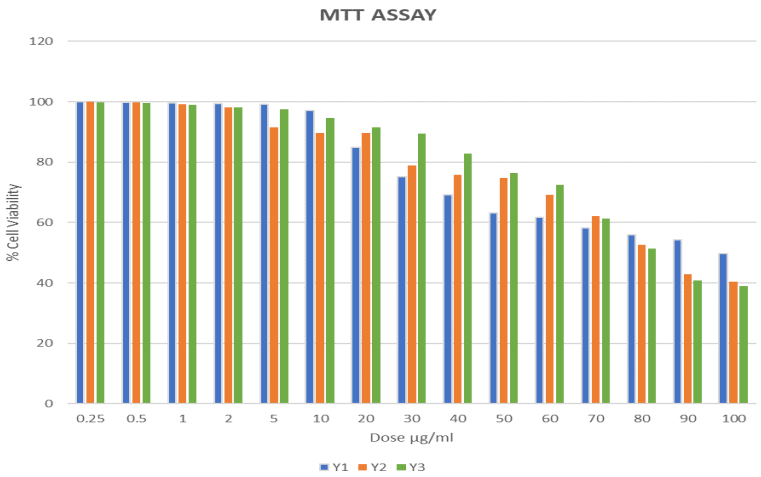

Fig 5: Viability of *vero* cells at different doses of different selenium sources.
Y1 (Blue bar) - Nano selenium derived from selenium powder.
Y 2 (Red bar) - Nano selenium derived from sodium selenite.
Y 3 (Green bar) - Organic selenium.

± 2.1 µg/ml. Salem *et al.* (2020) showed that IC-50 value of selenium nanoparticle against two different cell cultures, namely; human normal lung fibroblast (Wi 38) and human cancer colorectal adenocarcinoma epithelial (Caco-2) was 171.8 and 104.3 µg/ml respectively, data also proclaimed that the IC_{50} of Se-NPs for normal cell is higher than obtained from other cells. Hashem *et al.* (2021) showed that IC_{50} of mycosynthesized Se-NPs was 316.73 µg/ml towards *Vero* cell line CCL-81. Some reports declared that, Se-NPs showed lower cytotoxicity on normal cells compared with cancer cells (Vahidi *et al.* 2020).

Since, the synthesized nano-selenium had all the imperative characteristics of nano particles and our inclusion level of nano selenium in lamb feed is 0.3 mg/kg (NRC, 2007). Result of cell cytotoxicity assay moreover affirmed that both types of nano selenium and organic selenium (selenocysteine) are safe to use as lamb feed supplement and would replace its inorganic source to increase bioavailability and effectiveness.

CONCLUSION

Selenium nano particles would be successfully and effectively synthesized through wet chemical method from its precursor and the same could be characterized by several techniques as followed in this study for its quantity, size, shape, stability and purity.

The result of the present study showed that nano-selenium derived from both sources had all the characteristics of nano particle. Based on the result of *in vitro* cytotoxicity assay we would say that both type of nano selenium and organic selenium are nontoxic at our inclusion level. Hence, the synthesized selenium nano particles could be used as a feed supplement for lambs as per the standard recommended dosage (0.3 mg/kg of feed). However, systematic and rigorous research must be performed to assess if there are any negative effects after feeding animals for a prolonged period of time.

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