

## Effect of cerium doped Baddeleyite on their antibacterial activity by co-precipitation method

R.Mekala<sup>1\*</sup>, V. Rajendran<sup>2</sup> and B.Deepa<sup>2</sup>

<sup>1,\*2</sup> PG and Research, Department of Physics,  
Presidency College, Chennai-05, Tamil Nadu, India.

\*Corresponding author Email: [kalatamils@gmail.com](mailto:kalatamils@gmail.com)

**Abstract.** The present work, antibacterial activity of pure and Ceria doped Baddeleyite (ZrO<sub>2</sub>) nanoparticles (Nps) were synthesized by Co-precipitation method using different bacterial strains (gram positive and gram negative). The obtained samples were characterized by using XRD; SEM and antibacterial study. XRD and SEM confirmed the crystalline nature and morphology of the as synthesized products. The antibacterial studies revealed that doped sample exhibits higher activity compared to pure sample, which can be attributed to an increase of defect concentration (oxygen vacancy) in the sample. In addition, the possible formation mechanism of pure and Ce-doped ZrO<sub>2</sub> Nps have also been proposed.

**Keywords:** Baddeleyite, Ce-doped ZrO<sub>2</sub>, Antibacterial Activity, Morphology

### 1. Introduction

Nanostructured materials have promised several break-through applications in the Field of nano medicine and biomedical sciences due to their physical and chemical properties. Among nanostructured materials, metal oxides have emerged as a class of materials which is increasingly being studied for health-related applications<sup>1</sup>. Aside from various metal oxides, Baddeleyite (ZrO<sub>2</sub>) is recognized as an important ceramic material, used in many industrial applications such as high temperature solid oxide fuel cells (SOFC), high temperature pH sensors, oxygen sensor, refractory materials, capacitors, catalyst substrates, and as abrasives

<sup>2-5</sup>. ZrO<sub>2</sub> is also being an important material in the field of medicine because of good mechanical properties, and biocompatibility for aesthetic reasons<sup>6</sup>. When ZrO<sub>2</sub> is doped by rare earth metals, it shows an outstanding structural stability, high thermal conductivity and irradiation stability<sup>7</sup>. Many rare earth elements have been tried, in the past, to be implanted to ZrO<sub>2</sub>, to improve its optical properties, but few paper of the transition element have been concerned with antibacterial property of Nps implanted in the ZrO<sub>2</sub><sup>8-10</sup>. Amid from many rare earth element Ceria Nps is less toxic and have wide range of engineering and biological application<sup>11-14</sup>. Recently, Ce Nps have emerged as a fascinating material in biomedical science due to their ionic radii and ability to switch oxidation states between III and IV based on environmental conditions<sup>15</sup>. Nano ceria with the ionic radii for Ce<sup>3+</sup> is (1.14 Å), for Ce<sup>4+</sup> (0.97 Å) and the switch between oxidation state is comparable to that of biological antioxidants<sup>16</sup>. Newly, researchers have begun to investigate the antibacterial property of Ceria and Ce-doped materials and their use in biological applications<sup>17</sup>. To our knowledge, there is no much work has been done on the antibacterial activity of ZrO<sub>2</sub> Nps.

Recently, a variety of methods based on wet chemical routes have been extensively employed to synthesis of ZrO<sub>2</sub> Nps, the Co-Precipitation method is a simple, cost- effective, gives high purity



products, non-toxic and there is no need for any special instruments<sup>18,19</sup>. The present study aims to investigate the influence of the tetravalent Ceria doped ZrO<sub>2</sub> Nps on its structural, morphology, optical property and antibacterial activity Nps.

## 2. Experimental

### 2.1 Synthesis of Ce-doped ZrO<sub>2</sub> Nps

Ceria-doped ZrO<sub>2</sub> Nps were prepared by co-precipitation method using urea (NH<sub>2</sub>CONH<sub>2</sub>) as the precipitator. The reagents are Zirconyl nitrate hydrate ZrO (NO<sub>3</sub>)<sub>2</sub>.xH<sub>2</sub>O and Cerium (III) Nitrate Hexahydrate CeN<sub>3</sub>O<sub>9</sub>.6H<sub>2</sub>O. The initial compositions are 0.2 mole of ZrO (NO<sub>3</sub>)<sub>2</sub>.xH<sub>2</sub>O and 0.012 mole of CeN<sub>3</sub>O<sub>9</sub>.6H<sub>2</sub>O. First, the reagents together were dissolved in methanol (150ml) under stirring for 1 hr. Then urea was added into the above colloidal solution. Then the solution of the mixture was dried at 100°C for 24 hrs in a muffle furnace until the white powders were obtained. Subsequently, the obtained powder was calcined for 600°C for 6hrs respectively.

### 2.2 Characterization Technique

X-ray diffraction study was carried out for the sample by X-PERT PRO Diffractometer operated at 40kV with a current of 30mA using Cu-Kα (λ=0.1540nm), employing the scanning rate of 0.02°/s in the 2θ ranging from 20-70°. Surface morphology of the ZrO<sub>2</sub> nano samples were examined by SEM on a JOEL JSM 6390 Scanning Electron Microscope operated at an accelerating voltage at 15kV.

### 2.3 Evaluation of antibacterial activity of Ce-doped ZrO<sub>2</sub> Nps

The antibacterial activity of pure and Ce-doped ZrO<sub>2</sub> Nps were examined under two Gram Positive bacterial pathogens such as (*S. aureus*, *B. subtilis*) and two Gram Negative bacterial pathogens such as (*K. pneumoniae*, *P. aeruginosa*) by Agar Diffusion Method. These bacterial strains were grown in nutrient broth at 37°C for 24hrs or until visible growth was established. The antibacterial activity was demonstrated by the diameter of the zone of inhibition developed in and around the sample. Zone of inhibition is the area in which the bacterial growth is stopped due to the bacteriostatic effect of the compound and it measures the inhibitory effect of the compound towards particular microorganisms.

## 3. Result and Discussion

### 3.1 XRD analysis

The XRD patterns of pure and Ce-doped ZrO<sub>2</sub> precursors were shown in Fig.1. For all the precursors, the broadened humps are observed whether ceria doping or not, indicating their amorphous nature. The result demonstrates ceria doping has no obvious influence on the phase structure of the precursor. In spite of the samples, the crystal planes can be indexed to a zirconia phase with monoclinic (JCPDS No: 37-1484) and tetragonal (JCPDS No: 79-1769) structures. However, from Fig 1-a shows the m-ZrO<sub>2</sub> but in Fig 1-b shows there was shifting takes place from m-ZrO<sub>2</sub> to t-ZrO<sub>2</sub>. Moreover, the crystallite size of nano powders was calculated by Debye Scherer's equation,

$$D = K\lambda / \beta \cos\theta \rightarrow (1)$$

The specific surface area of the as synthesized samples were calculated by using the formula,

$$S = 6 / D * \rho \rightarrow (2)$$

Where, S – Specific Surface area (m<sup>2</sup>/g), D – average particle size, ρ – density of ZrO<sub>2</sub> (g/ cm<sup>-3</sup>) and both the calculated values are shown in Table 1.

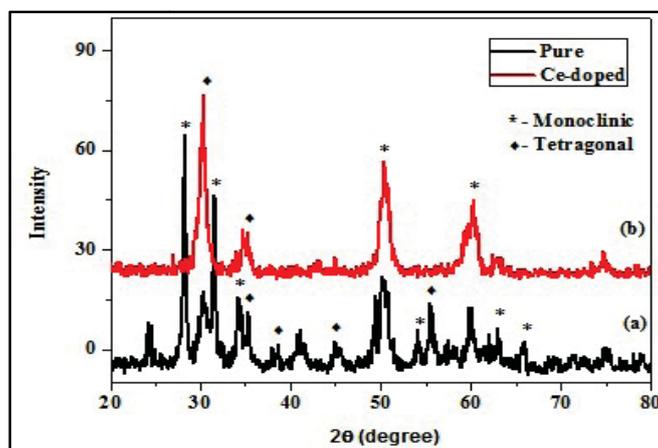


Fig. 1. XRD patterns of a) Pure  $ZrO_2$  and b) Ce-doped Zirconia Nano crystallites.

Powder	Crystallite Size (nm)	Specific Surface Area ( $m^2/g$ )
Pure	12.09	87.37
Ce-doped	8.52	123.98

Table 1 Crystallite Size of nano-sized Zirconia by XRD analysis.

### 3.2 SEM analysis

SEM analysis is employed to visualize the size and shape of the pure and Ceria-doped  $ZrO_2$  Nps. SEM micrographs of both pure and doped  $ZrO_2$  Nps under different magnifications are shown in Fig. 2 (a-b). From Fig.2. (a-b) the particles observed are clumpy agglomerated and spherical in shape with smooth and fused surface. The unvarying size distribution of the particles may be due to the sintering process which allows the growth by aggregation of particles through their grain boundaries.

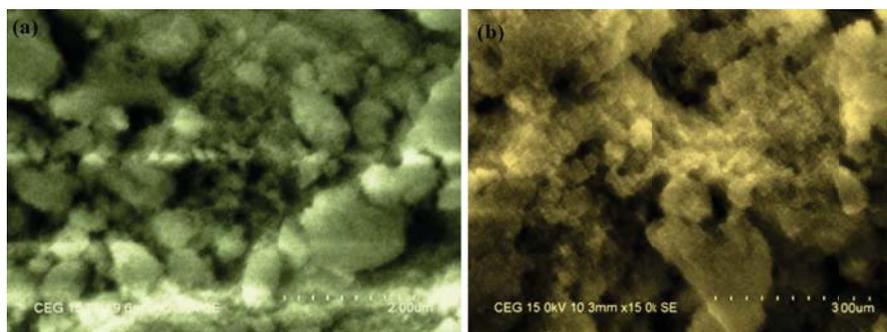
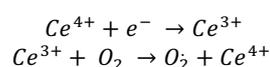


Fig.2. SEM micrograph of a) Pure  $ZrO_2$  b) Ce-doped  $ZrO_2$  by Co-Precipitation Method.

### 3.3 Antibacterial Assessment of Pure and Ce-doped $ZrO_2$ Nps

In the present Study, pure and Ce-doped  $ZrO_2$  Nps are studied extensively to explore their utility as a potential antibacterial agent. The antibacterial activity has been studied against (G+) bacteria such as

(*S. aureus*, *B. subtilis*) and (G-) bacteria such as (*K. pneumoniae*, *P.aeruginosa*) using the concentration 50µg/ml. Both pure and doped sample pronounced significant growth inhibitory effect against both (G+) and (G-) bacteria due to their large surface area by their nano size which was shown in Fig.3. Zone of inhibition values determined for the pure and doped samples was shown in Table.2. The antibacterial property shows more efficient for Ce-doped ZrO<sub>2</sub> Nps compared to pure ZrO<sub>2</sub> in (G+) bacteria (i.e. *S. aureus*) than in (G-) bacteria which are clearly visualized in the antibacterial photographs. This difference in antibacterial performance may be due to the following reasons: the Ce-doping may enhance the ROS generation capability of ZrO<sub>2</sub> Nps. In the case of Ce-doped ZrO<sub>2</sub> sample, the probability of electron-hole pair recombination may be reduced due to the trapping of electrons by Ce ions by the following process<sup>20</sup>.



The mechanisms of the antibacterial activity of ions are not fully explained. However, the literature provides information on three hypothetical mechanisms<sup>21</sup> (Figure.4). Firstly, ceria and zirconia ions are penetrating into the bacterial cell; it will lead to affect the production of intracellular ATP. When the ATP production is started to affect, it will tend to disrupt the process of DNA replication. Secondly, amassing of ions in the cell membranes of bacteria, and thus with changes in their permeability. The transportation of protons through the cell membrane is prohibited. Hence, it leads to the destruction of the cell membrane and the death of the bacterial cell. Thirdly, the ion induction of reactive oxygen species (ROSs). Oxygen radicals are able to react with the components of the membrane, cell wall of bacteria, and as well as on other cell components (e.g., mitochondria), causing irreversible changes in their structure and thus the death of the bacterial cell.

Hence, the augmentation in the antibacterial activity of ZrO<sub>2</sub> Nps with Ce doping may be attributed to the electrons trapped in the Ce sites. The trapping electrons will lead to more ROS generation, resulting in higher antibacterial activity of Ce-doped ZrO<sub>2</sub> Nps. From the above probable discussions we clearly came to know about the enhanced antibacterial activity of pure and doped ZrO<sub>2</sub> Nps.

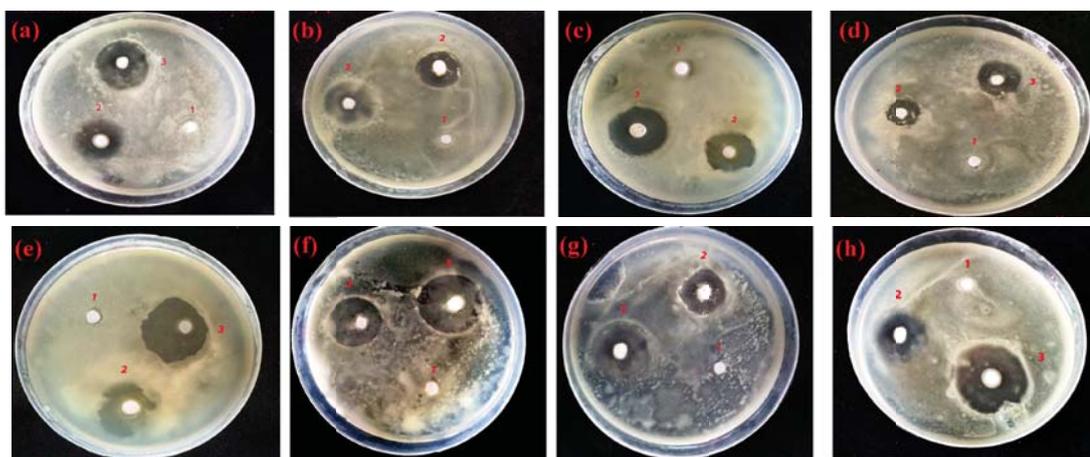


Fig. 3. Antibacterial activity of pure and Ce - doped ZrO<sub>2</sub>Nps against (G+) bacterial pathogens such as (i)*S.aureus*(ii)*B.subtilis*(a,b) Pure and (e,f) Ce-doped ZrO<sub>2</sub>and (G-) bacterial pathogens such as (i) *K.pneumoniae* and (ii) *P.aeruginosa* (c,d) Pure and (g,h) Ce-doped ZrO<sub>2</sub> nanomaterials

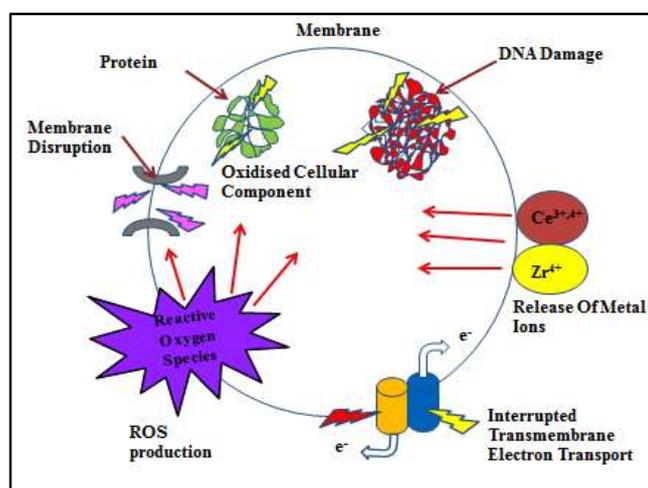


Fig.4. Antibacterial mechanism of Nps and their ions, adapted from with permission.

Samples	Zone of Inhibition (mm)			
	Bacterial Pathogens			
	Gram Positive		Gram Negative	
	Bacillus Subtilis	Staphylococcus Aureus	Klebsiella Pneumonia	Pseudomonas Aeruginosa
Pure	14	13	12	8
Ce-doped	15	16	15	11

Table 2 Zone of inhibition (mm) values against test organisms.

#### 4. Conclusion

In summary, the pure and Ce-doped  $ZrO_2$  Nps via a simple chemical co-precipitation technique. The XRD results have shown the formation of monoclinic and tetragonal structure of  $ZrO_2$  for all samples. The SEM images have revealed the formation of spherical like morphology for pure and doped sample. The Ce-doped  $ZrO_2$  Nps have been observed to be more effective than the pure  $ZrO_2$  Nps both against (G+) i.e., *S. aureus* and (G-) such as *K. pneumonia* bacteria. Interestingly, it has been found that *S. aureus* bacterium can be completely eradicated by application of Ce-doped  $ZrO_2$  Nps.

#### Acknowledgement

The author is grateful to the Department of Science and Technology (DST) for extending financial assistance to carry out this work.

#### References

- [1] Gu F X, Karnik R, Wang A Z, Alexis F, Nissenbaum E L, Hong S, Langer R S and Farokhzad O C, 2007 *Nano today* **2** 14.
- [2] Patil K C, Hedge M S, Rattan T, Aruna S T, 2008 *World Scientific*, ISBN-13 978-981-279-314.

- [3] Li Z, Lee W E, and Zhang S, 2007 *J. Am. Ceram. Soc.*, **2** 364.
- [4] Mucillo R, Buissa Netto R C, and Mucillo E N S, 2001 *Matter. Lett.* **49** 197.
- [5] Costa G C C and Muccillo R, 2008 *Solid State Ionics*, **179** 1219.
- [6] Martin Stefanic, Kristoffer Krnel, Tomaz Kosman, Engineering Ceramic Department, JJ. S. Institute, Ljubljana, Slovenia.
- [7] Aytimur A, Uslu I, Kocyigit S, Ozcan F, 2003 *Ceramics International*, **38** 51.
- [8] Feng Gu, Shu Fen Wang, Meng Kai Lu, Guang Jun Zhou, Su Wen Liu, Dong Xu, Duo Rong Yuan, 2003 *Chemical Physics Letters* **380** 185.
- [9] Masonori Hirano, Hayato Dozono, 2013 *Journal of Solid State Chemistry* **204** 355.
- [10] Ignacio Azocar, Esteban Vargas, Nicole Duran, Abel Arrieta, Evelyn Gonzalez, Jorge Pavez, Marcelo J. Kogan, Jose H. Zagal, Maritza A. Paez, 2012 *Materials Chemistry and Physics* **137** 396.
- [11] Tarnuzzer R W, Colon J, Patil S, Seal S, 2012 *Nano Lett.* **5** 2573.
- [12] Chen J, Patil S, Seal S, McGinnis J F, 2012 *Nat. Nanotechnology*. **1** 142.
- [13] Cai X, Sezate S A, Seal S, McGinnis J F, 2012 *Biomaterials* **33** 8771.
- [14] Das M, Patil S, Bhargava N, Kang J F, Riedel L M, Seal S, 2007 *Biomaterials* **28** 1918.
- [15] Karakoti A S, Kuchibhatla S V N T, Babu K S, Seal S, 2007 *J. Phys. Chem. C* **111** 17232.
- [16] Korsvik C, Patil S, Seal S, Self W T, 2007 *Chem. Commun.* **10** 1056.
- [17] Das S, Singh S, Dowding J M, 2012 *Biomaterials*, **33** 7746.
- [18] Gambhire A B, Lande M K, Kalokhe S B, Shirsat M D, Patil K R, Gholap R S, Arbad B R, 2008 *Materials Chemistry and Physics* **112** 719.
- [19] Geethalakshmi K, Prabhakaran T and Hemalatha J, 2012 *World Academy of Science, Engineering and Technology*, **6** 04.
- [20] Tariq Jan, Javed Iqbal, Qaiser Mansoor, M Ismail, M Sajjad Haider Naqvi, Asmagul, S. Faizan-Ul-Hassan Naqvi and Fazal Abbas, 2014 *Journal of Physics D: Applied Physics* **47** 355301.
- [21] Joanna Kolmas, Ewa Groszyk, and Dagmara Kwiatkowska-Rozycka, 2014 *Hindawi Publishing Corporation Biomed Research International*, Article ID 178123, **15** pages, <http://dx-doi.org/10.1155/2014/17812>.