Original Article

Synthesis, characterization and antibacterial activity of cobalt doped cerium oxide (CeO₂:Co) nanoparticles by using hydrothermal method

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Different concentrations (2, 4, 6, and 8 mole %) of cobalt doped cerium oxide nanoparticles (CeO₂:Co NPs) were synthesized by hydrothermal method. The synthesized samples were characterized by using various techniques to understand their structural, optical and surface morphological properties. The face-centred cubic (FCC) structure of the CeO₂:Co NPs was identified from the X-ray diffraction (XRD) analysis. The calculated crystallites size of the CeO₂:Co NPs were decreased from 20 nm to 17 nm on increased the concentration of cobalt from 2 mole % to 8 mole %. The bonding formation between cerium and oxygen (Ce—O) was confirmed using Fourier transform infra-red spectroscopy (FTIR). The surface morphology and shape of the CeO₂:Co NPs were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The SEM images have revealed a cube shaped, uniformly distributed and well dispersed CeO₂:Co NPs. Further, a slight distortion of surface morphology was obtained when increased the concentration of cobalt. The optical properties were investigated by using ultra-violet visible (UV–vis) spectroscopy. A optical absorption band nature was observed from CeO₂:Co NPs when compared with bulk spectrum. The bandgap energy of the CeO₂:Co NPs were increased from 3.64 eV to 3.69 eV on increased the cobalt concentration. The photoluminescence (PL) emission spectrum of CeO₂:Co NPs showed an enhanced defect of reduced emission by using spectrofluorometer. The CeO₂:Co NPs resulted good antibacterial activity against pathogenic bacteria such Escherichia coli, Staphylococcus aureus, Bacillus Cereus and Salmonella Typhi. Hence, the CeO₂:Co NPs could be used as biomaterial in nano-biotechnology applications.

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1. Introduction

In recent years, the design, synthesis and characterization of cerium oxide (CeO$_2$) nanoparticles have drawn the attention of researchers due to their peculiar properties in the field of material science and technology. It is an attractive material which is used in various industrial products such as polishing agents, sun screens, fuel cells, photocatalysts and sensors applications [1–3]. CeO$_2$ nanostructures have attracted extensive attention on the electronics and biological applications such as device fabrications, anti-oxidant activity, anti-bacterial activity and anti-cancer activities, which depends on the particle size, morphology and biocompatible nature. For the synthesis of CeO$_2$ nanostructures, various chemical synthesis methods such as co-precipitation, sol–gel, chemical vapour deposition and hydrothermal techniques are used to fabricate CeO$_2$ nanostructures with controlled morphology [4–8]. Among these techniques, the hydrothermal method was getting more important, because of efficient synthesis process and morphology controlled growth. Moreover, the CeO$_2$ nanoparticles played an important role in the remediation of toxicity against microorganisms of bacteria, yeast and fungi [9]. Antibacterial activity of transition metal doped various metal oxide nanostructures has been reported to an electrostatic induction nature between nanoparticles and bacteria cell membrane [10–12]. Therefore, in the present research work, we have synthesized cobalt (Co) doped cerium oxide (CeO$_2$) nanoparticles by using hydrothermal method and their structural, optical, morphology and anti-bacterial activity results were discussed in detail.

2. Materials and methods

2.1. Reagents

Cerium nitrate Ce(NO$_3$)$_3$, cobalt chloride (CoCl$_2$) and trisodium phosphate Na$_3$PO$_4$ were purchased from Merck products, Mumbai, India. All the chemicals were used without purification. The double distilled water was used as solvent to prepare the solutions.

2.2. Synthesis of cobalt doped CeO$_2$ nanoparticles

Cobalt doped cerium oxide nanoparticles were synthesized using cobalt chloride and cerium nitrate as precursors. A 0.02 mole of trisodium phosphate solution (20 ml) was slowly added dropwise to 0.1 mole of cerium nitrate solution (60 ml) under constant stirring condition. During the synthesis, 2, 4, 6 and 8 mole % cobalt chloride solution was added to the above reaction mixture solution. The reaction was allowed for 30 min at same condition resulted white colloidal solution. The residue was transferred to the suitable autoclave container and the hydrothermal treatment carried out at 180 °C for 15 h. After the reaction, the autoclave container was brought to room temperature. Then, the colloidal was separated by using centrifugation and the obtained product was thoroughly washed with double distilled water followed by ethanol and dried at 80 °C for 4 h. For comparison purpose, the pure cerium oxide nanoparticles were synthesized by using the same procedure without cobalt.

2.3. Characterization

Transformation of functional group present in the cerium oxide and cobalt doped cerium oxide nanoparticles were recorded using FTIR spectrophotometer (FTIR, Brucker-Tensor 27). The crystalline nature of the synthesized nanoparticles was analyzed by using X-ray diffractometer (XRD, Shimadzu-6000). The absorption of the nanoparticles was measured using spectrophotometer (UV–vis, Jasco V530). The emission spectra of the nanoparticles were measured using spectrofluorometer (Horiba Jobin, Fluoromax-4). The formation of nanoparticles size and surface morphology of the nanoparticles were studied by scanning electron microscope (SEM, JOEL JSM-6390) and transmission electron microscope (TEM, Tecnai F-12).

2.4. Antibacterial activity

The antibacterial activity of pure cerium oxide and cobalt doped cerium oxide nanoparticles were tested against Escherichia coli, Staphylococcus aureus, Bacillus Cereus and Salmonella Typhi using disc diffusion method. The pure CeO$_2$ and Co doped CeO$_2$ nanoparticles were prepared in appropriate concentration of 1 mg/ml with dimethylsulfoxide solution for this process. Then, the dispersed nanoparticles were impregnated to each sterile disc by using micro-pipette. After that the discs were kept on culture swapped Mueller Hinton Agar medium using sterile force and allowed to incubate for 24 h. The average zone of inhibition diameter was measured in millimetre (mm).

3. Result and discussion

3.1. FTIR study

The FTIR spectra of the pure CeO$_2$ NPs and Co doped CeO$_2$ NPs were shown in Fig. 1. The transmittance peaks obtained below the range of 700 cm$^{-1}$ [13], including the presence of peaks at 475, 545, 615 cm$^{-1}$ is attributed due to the Ce–O stretching mode and it confirms the formation of CeO$_2$ structure. Further, the transmittance peaks obtained at 715, 735, 950 and 1052 cm$^{-1}$ is attributed to CO$_2$ asymmetric stretching vibration and CO$_2$ bending vibration, and C–O stretching vibration. In addition, the peak at 818 cm$^{-1}$ with the O–C–O was absorbed due to the stretching frequencies. In addition, the band at 1381 cm$^{-1}$ was due to N–O stretch formation of oxygen present in cerium with nitrogen and the band at 1629 cm$^{-1}$ corresponds to the bending of H–O–H, which was partly overlapping the O–C–O stretching band [14]. The band at 2427 cm$^{-1}$ was due to the presence of dissolved or atmospheric CO$_2$ in samples and the absorption band at 3410 and 3500 cm$^{-1}$ was due to the OH stretching vibration/physical absorbed H$_2$O/surface OH groups [15]. Based on previous report, a weak band around at 2037 cm$^{-1}$ was attributed to CO bonded to metallic fraction of Co ions [16] and therefore,
we believed that it was occurred in Co doped CeO₂ nanoparticles also. Moreover, the N–O stretches and H–O–H bending nature were getting strengthened when increasing the concentration of Co doping in steps of 2, 4, 6 and 8 mole %. Then, these observations show the bonding nature of Co ions with CeO₂ nanoparticles. Hence, the chemical structure and other functional groups were confirmed.

### 3.2. XRD study

The XRD patterns of the pure CeO₂ NPs and Co doped CeO₂ NPs were shown in Fig. 2. The XRD patterns are in tremendous concurrence with standard values of CeO₂ (JCPDS card no. 43-1002). According to distinct diffraction peaks in the XRD patterns were clearly indicates face-centred cubic structure. The obtained 2θ values of 28.5, 33.0, 47.4, 56.3, 59.0, 69.4, 76.7 are corresponding to the (1 1 1), (2 0 0), (2 2 0), (3 1 1), (2 2 2), (4 0 0), (3 3 1) planes and it is also similar to the previous reports [17,18]. In the CeO₂:Co NPs, the lack of Co²⁺ or Co ions may be diluted in the CeO₂ and, therefore, the Co peaks were not appeared. However, the crystallities size was slightly decreased when increasing the concentration of Co doping in steps of 2, 4, 6 and 8 mole %, while the (1 1 1) plane takes a gradual peak shift towards lower angle side [19,20]. Actually, the ionic radii of Ce³⁺ ions has larger size of 1.03 Å than smaller size ionic radii of Co³⁺ ions has 0.74 Å and, therefore, the Co ions were possible to substituted in the CeO₂ crystal lattice [21]. Hence, the peak broaden was increased due to the decreased particles size [8,22] as shown in Fig. 3. The crystalline sizes or average particles sizes were calculated using Debye-Scherrer’s formula [23] and their sizes were 17–20 nm with d-spacing values of 3.14–3.08 Å. The calculated crystalline sizes and their corresponding d-spacing values are given in Table 1.

### 3.3. SEM analysis

The SEM images of the pure CeO₂ NPs and Co doped CeO₂ NPs were shown in Fig. 4. A cube shaped surface morphology was observed from pure CeO₂ NPs and from the CeO₂:Co NPs showed a slight distortion in the surface morphology on increasing the cobalt doping concentration, which has been reported earlier [24]. The incorporation of cobalt doping was indicated by small leaves or feathers like structures on the surface of the cube shaped CeO₂ NPs. The slight alteration on the shape of CeO₂ strongly indicates the effect of Co ions in the CeO₂ crystal lattice, which made an agreement with the XRD results of average particles size. Further, the EDAX spectrum was recorded to know the chemical composition of Ce, O and Co ions distributions in the CeO₂ NPs, shown in Fig. 5. The observed result indicates the chemical composition nature and also confirms the doping effect of cobalt ions. Moreover, the TEM images were recorded to observe the particles size and particles distribution. From that, a well grown nanoparticle was observed with well distributed nature shown in Fig. 6. However, the surface morphology was not clear, because of the embedded nature of particles in the copper grid.

### 3.4. Optical studies

UV absorption spectra of the pure CeO₂ NPs and Co doped CeO₂ NPs were shown in Fig. 7. The pure CeO₂ NPs show a strong absorption at 345 nm and their corresponding bandgap energy (E₉) value was 3.62 eV, which was calculated using the E₉=1242/λ(absorption) relation. In the CeO₂:Co NPs, the
Table 1 – XRD content for undoped and Co doped CeO₂ nanoparticles.

<table>
<thead>
<tr>
<th>Samples</th>
<th>2θ (degree)</th>
<th>FWHM (radians)</th>
<th>Crystallite size (nm)</th>
<th>d-Spacing (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CeO₂</td>
<td>28.90</td>
<td>0.43</td>
<td>19.9</td>
<td>3.0869</td>
</tr>
<tr>
<td>CeO₂:Co (2 M%)</td>
<td>28.59</td>
<td>0.44</td>
<td>19.44</td>
<td>3.1197</td>
</tr>
<tr>
<td>CeO₂:Co (4 M%)</td>
<td>28.77</td>
<td>0.46</td>
<td>18.6</td>
<td>3.1005</td>
</tr>
<tr>
<td>CeO₂:Co (6 M%)</td>
<td>28.38</td>
<td>0.49</td>
<td>17.4</td>
<td>3.1423</td>
</tr>
<tr>
<td>CeO₂:Co (8 M%)</td>
<td>28.47</td>
<td>0.50</td>
<td>17.1</td>
<td>3.1325</td>
</tr>
</tbody>
</table>

Fig. 4 – SEM images of (a) undoped CeO₂, (b) 2 M% Co, (c) 4 M% Co, (d) 6 M% Co and (e) 8 M% Co doped CeO₂ nanoparticles.

absorption intensity of CeO₂ nature was quenched [25], which was maximum at visible region when increasing the Co doping concentration from 2 mole % to 8 mole % shown in Fig. 8. As the mean time, it may be the distributed nature of Co ions otherwise un-reacted nature of some Co doping ions. Therefore, the obtained bandgap energy value was slightly increased to 3.67 eV and it is believed to be the decreased particles size [26,27]. Hence, the enhanced bandgap energy was due to the charge-transfer from O²⁻ to Ce⁴⁺ energy levels in CeO₂ crystal lattice [28]. The calculated absorption wavelengths and their corresponding bandgap energy values are given in Table 2.

3.5. Photoluminescence

The PL emission spectra of the pure CeO₂ NPs and Co doped CeO₂ NPs were shown in Fig. 9, using the excitation wavelength of 340 nm corresponding to the absorption spectrum values. The pure CeO₂ NPs exhibit a strong emission peak
at 375 nm, it was related to the transition of electron from localized Ce_{4f} state to O_{2p} of valence band. In addition, an emission peak obtained at 392 nm was originated from defect states widely existing between Ce_{4f} states to O_{2p} of valence band [21]. Moreover, an emission of CeO_{2}:Co NPs was gradually decreased around at 400 nm and also it extended up to 550 nm. The localized Ce_{4f} state to O_{2p} of valence band in CeO_{2}:Co NPs related to the emission, which was gradually decreased in the CeO_{2} crystal lattice due to doping effect of Co ions. On the other hand, the emission band was gradually increased in the range of 400 nm to 450 nm and also it was extended up to 550 nm. Meanwhile, the emission nature was red-shifted, because of reduced defect levels caused by the Co ions. Hence, the emission intensity of CeO_{2} nature got gradually quenched [29] and it was due to the formation of more oxygen defects or increases of surface defects and suppresses of electron transfer from Ce_{4f} to O_{2p} levels [21,30] when increasing the Co doping concentration from 2 mole % to 8 mole %.

### 3.6. Antibacterial activity

The antibacterial activity pure CeO_{2} NPs and Co doped CeO_{2} NPs were tested against four pathogenic bacteria's of E. coli, S. aureus, B. cereus and S. typhi. The antibacterial result of the pure CeO_{2} NPs and Co doped CeO_{2} NPs were shown in Fig. 10. The Co doped CeO_{2} NPs exhibits a higher zone of inhibition (ZOI) as compared with pure CeO_{2} NPs. These observations indicate that the interaction between CeO_{2}:Co NPs and bacteria cell membrane induces the toxicity to bacteria and caused cell death. Both the pure CeO_{2} NPs and CeO_{2}:Co NPs shows a good death effect against all the bacteria. The zone of inhibition was increased as the doping concentration of Co was increased. Hence, the comparison of antibacterial activity with different bacteria was shown in Fig. 11 and their zones of inhibition results are given in Table 3. Interestingly, the CeO_{2}:Co NPs shows an enhanced antibacterial activity as compared with the previously reported on Co doped metal oxide nanoparticles for antibacterial and anticancer activities [31,32]. Among the four concentrations (2, 4, 6 and 8 mole %), the 8 mole % of CeO_{2}:Co NPs showed higher zone of inhibition against
E. coli (24 mm), B. cereus (27 mm), S. aureus (23 mm) and S. typhi (25 mm). It was believed to be the electrostatic interaction between positive charged nanoparticles and negative charged bacteria, which leads the small-sized nanoparticles penetration inside the cell wall and cause the cell damage. While the generation of reactive oxygen species (ROS), large surface area or small-sized particle nature and the release of constituent ions through efflux mechanism [33]. Therefore, the antibacterial activity of the CeO$_2$:Co NPs were found an enhanced rate.

Fig. 6 – TEM images of (a) undoped CeO$_2$, (b) 2 M% Co, and (c) 8 M% Co doped CeO$_2$ nanoparticles.

Fig. 7 – UV absorption spectra of (a) undoped CeO$_2$, (b) 2 M% Co, (c) 4 M% Co, (d) 6 M% Co and (e) 8 M% Co doped CeO$_2$ nanoparticles.

Fig. 8 – UV Bandgap energy values of undoped CeO$_2$, 2 M% Co, 4 M% Co, 6 M% Co and 8 M% Co doped CeO$_2$ nanoparticles.

Fig. 9 – PL emission spectra of (a) undoped CeO$_2$, (b) 2 M% Co, (c) 4 M% Co, (d) 6 M% Co and (e) 8 M% Co doped CeO$_2$ nanoparticles.

4. Conclusions

The pure CeO$_2$ and Co doped CeO$_2$ were successfully synthesized by using hydrothermal method. From XRD patterns, a face-centre cubic crystal structure was confirmed, and their crystalline sizes were calculated approximately 17–20 nm.
FTIR spectrum shows the Ce—O chemical bonding nature and the presence of functional groups were confirmed. From SEM images, a cubic shaped surface morphology was observed. From UV optical spectrum, a blue-shifted absorption nature was observed, and their calculated bandgap energy values were approximately 3.62–3.67 eV. The PL emission spectrum deals a strong blue emission nature and a reduced defect level was observed due the doping of Co ions. Further, the antibacterial activity of Co doped CeO₂ nanoparticles were studied against different types of bacteria. An enhanced anti-bacterial activity killing effect was observed due to the increased concentration of Co doping from 2% to 8%.

**Conflicts of interest**

The authors declare no conflicts of interest.

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