Oxidase-like activity of magnetically separable nano ceria for catechol detection

Suraj V. Yadav¹ · Virendra K. Rathod¹

© Springer Nature Switzerland AG 2019

Abstract

The present work demonstrates the use of novel properties of ceria magnetic nanoparticles ($\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs) as oxidase mimetics to detect catechol. The $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs was prepared by simple co-precipitation method. The prepared nanoparticles were confirmed by different techniques such as FTIR, XRD, SEM-EDS, and XPS. $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs exhibits similar activity as tyrosinase enzyme to catalyse the oxidation of colorless catechol to colored quinone complex, which provides colorimetric detection of catechol. Characterization techniques show that the oxidase activity of the $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs initiates due to $\text{Ce}^{4+}/\text{Ce}^{3+}$ and quinone complex formation by catechol ligand. In addition, $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs can be easily separated from the reaction solution by using a magnet and reused for the next cycle.

Keywords Ceria magnetic nanoparticles · Enzyme mimic · Catechol detection · Quinone complex · Nanozyme

1 Introduction

Natural enzymes are used in the analysis of biological and chemical substances, food processing, and in biofuel production. It is also widely used in the synthesis of pharmaceuticals and fine chemicals due to their high substrate specificities and high catalytic efficiency under mild conditions [1, 2]. However, they also have inherent restrictions such as expensive techniques for preparation and purification, instability affected by denaturation, and inadequacy for large-scale applications [2, 3]. Therefore, with developments in artificial enzyme, the replacement of enzyme by other material is possible, as they not only have benefits with respect to cost, easy storage, and stability but also provide high substrate specificities [4–6].

Ceria metal nanoparticles (NPs) have received significant attention in latest years [7–10] because of their special properties due to large surface area [10, 11], quantum confinement, and super paramagnetism, which are very dissimilar from the properties demonstrated by the identical materials in bulk form. Fe_3O_4 magnetic nanoparticles

demonstrate an enzyme mimic activity, analogous to naturally peroxidases [3]. A variety of inorganic nanomaterials have been established as enzyme mimics [12–17], accompanied by transition and inner-transition metal oxides (CeO_2 , CuO, FeS, CuS, and FeSe), carbon-based nanomaterials and noble metals (Au and Pt) [1, 2, 18]. One area of intense scientific interest is nanoparticles that show enzyme-like activity [3, 19–21]. Even though natural enzymes show good catalytic activity and selectivity, they can be destabilized by environmental conditions [18, 22]. Purification and immobilization of natural enzymes can be costly and time-consuming [1, 18, 22], requiring complex and expensive technology such as chromatography and electrophoresis. In contrast, nanomaterials are stable, easily synthesized, inexpensive, have high catalytic efficiency, and selectivity.

Till date, a wide range of nanoparticles has been recognized as enzyme mimetics, containing metal oxides and carbon-based nanostructure. These nanomaterials seem to encompass an infinite number of potential structures; though, it is possible that they possess

✉ Virendra K. Rathod, vk.rathod@ictmumbai.edu.in | ¹Department of Chemical Engineering, Institute of Chemical Technology, Matunga (E), Mumbai 400019, India.

several unifying and classifying principles. Usually, the enzyme-like activities of nanomaterials can be divided into four categories: (1) peroxidase-like, (2) oxidase-like, (3) superoxide dismutases like (SOD) and (4) catalase like enzyme activities [1, 23, 26]. Other nanoparticles (NPs) such as CeO_2 and Co_3O_4 NPs are competitive candidates to substitute SOD. Sometimes single nanomaterial can show more than one enzyme-like property and those enzyme-like activities may change depending on external conditions. For example, nanomaterials containing precious metals, such as Au and Ag NPs, display peroxidase-like activities under acidic conditions, but catalase-like activities under alkaline conditions [4, 24]. Although significant efforts have been expended to develop enzyme-like nanomaterials over the past decade, most nanomaterials research has focused on achieving catalase-like and peroxidase-like activity [2]. Nanomaterials having oxidase-like activities has received less attention [1]. Polyphenol oxidases are of interest because of unique properties and their effects on the polyphenols extensively found in natural products, which have long been thought to be beneficial to human health. Biochemical investigations in 1895 considered the oxidation of phenolic compounds to quinones and their subsequent polymerization to be the major path for melanin formation. In humans, areas of increased accumulation of melanin in the skin or the redistribution of epidermal melanin can effect dark or light patches which may be considered aesthetically disagreeable. In plant materials, the polymerization of polyphenols causes the enzymatic browning of fruits and vegetables, resulting in altered nutritional and organoleptic properties, diminished storage life and product value [25]. Nevertheless, oxidations of polyphenols can be helpful and even considered essential to the quality of a product. In the processing of black tea, coffee, and cocoa, oxidation improves the quality of the beverages, making products more flavorful and providing distinctive organoleptic properties. As the oxidation of polyphenols has diverse and significant biological roles, the possibility that certain nanomaterials could display oxidase-like properties must be investigated and understood.

In this study, we report that $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs were synthesized and used for the first time by the colorimetric method for catechol detection by oxidation of *o*-diphenols to *o*-quinones. Also, the oxidase activity of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs was checked and compared with the tyrosinase enzyme. We have investigated these oxidation processes using UV-vis spectrophotometry. Our findings provide important insights, which can provide information on structural properties and future applications of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs. This will help in the detection of other polyphenolic compounds.

2 Materials and methods

2.1 Materials

Ammonium ceric nitrate, ferrous chloride ($\text{FeCl}_2\cdot 4\text{H}_2\text{O}$), ferric chloride (FeCl_3), ammonia solution, Ascorbic acid and catechol were purchased from HiMedia Laboratories Pvt. Ltd. Mumbai, India. Tyrosinase with 1000 U/mg activity was procured from Sigma Aldrich Pvt. Ltd, India. De-ionized (DI) water with conductivity of 0.055 microsiemens was used for all the experiments.

2.2 Methods

2.2.1 Fe_3O_4 Magnetic nanoparticle synthesis

Precipitation method was used for the synthesis of magnetic nanoparticles [26]. The ferrous ions and ferric ions were dissolved in 40 mL sonicated deionized water at a molar ratio of 1:2. Further the reaction was carried out in a 50 mL glass reactor with continuous stirring (100 rpm) at 50 °C. After 30 min of mixing, pH 12 was adjusted by adding NH_3 solution and further stirred for 30 min. Thereafter, resulting MNPs were centrifuged, washed and dried at 60 °C for 2 h.

2.2.2 Synthesis of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ nanoparticles

$\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs was prepared from ammonium ceric nitrate hexahydrate by precipitation method [27–29]. 0.2 g ammonium ceric nitrate hexahydrate was dissolved in 30 mL DI water, which was taken in a 50 mL glass reactor at 50 °C temperature with 100 rpm stirring and pH 12 was maintained by adding ammonia solution to precipitate ceria nanoparticles. Further, 1 g Fe_3O_4 NPs was added and the reaction was agitated for 1 h. The reaction mixture was centrifuged and washed with DI water. The collected precipitate was dried at 60 °C and Ce doped Fe_3O_4 NPs magnetically separable catalyst was synthesized.

2.2.3 Oxidase like activity

To study the oxidase mimicking activity of ceria magnetic nanoparticles, different concentrations of catechol (1–7 mM), $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs (1–9 mg) and phosphate buffer solution (0.05–1 M) with pH 7.8 was examined at various times intervals (5–20 min) at room temperature. On completion of the reaction, $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs was separated by an external magnet and checked for quinone complex formation by measuring absorbance using spectrophotometer [1]. The effect of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs concentration

was inspected in the range of 1–9 mg, using 5 mM catechol. Also, the optimization of time and ionic strength was studied by using 5 mg $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs with 5 mM catechol concentration. The effect of time was studied in buffer solutions for 5–20 min at room temperature and consequence of ionic strength was observed in buffer concentration changing from 0.05 to 1 M (pH 7.8). The absorbance was measured in the visible range. The absorbance of quinone complex due to tyrosinase enzyme was compared with $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs generated quinone complex.

The interference study for catechol detection has been carried out by adding 5 mM ascorbic acid in the solution containing catechol (5 mM) and $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs (5 mg), the absorbance of the colored solution was measured and compared with the ascorbic acid solution.

2.2.4 Characterization of prepared $\text{CeO}_2\text{-Fe}_3\text{O}_4$ nanoparticles

The prepared ceria magnetic nanoparticles ($\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs) were characterized, using Fourier transform infrared spectra (FTIR) recorded over 400–4000 cm^{-1} on a Shimadzu IR affinity—1S spectrometer using DRS 8000 accessory. The crystal structure of the ceria magnetic nanoparticles was studied using X-ray diffraction (XRD) ($2\theta = 5^\circ\text{--}80^\circ$, Bruker AXS powder diffractometer, D8-Advance). The XRD analysis was done by scanning the sample within a range of 5–80 2θ (degree) for 2 h, using monochromatized Cu K α radiation (1.54 Å). The uniform distribution of ceria magnetic nanoparticles was determined by using elemental mapping analysis. This was also further supported by scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM–EDX), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) Analysis.

3 Results and discussion

3.1 Characterization of magnetically separable ceria ($\text{CeO}_2\text{-Fe}_3\text{O}_4$) NPs

The FTIR spectra of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ magnetic nanoparticles is shown in Fig. 1. The spectrum of the $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs shows a peak in the range of 500–600 cm^{-1} which is metal–oxygen stretching vibration in the tetrahedral site [27].

XRD pattern of the synthesized $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs is shown in Fig. 2. According to JCPDS card number 01-075-0449 database, the peak at $2\theta = 35.64^\circ$ recognized to (1 1 0) plane of Fe_3O_4 . They have rhombohedral structure and sharp reflections indexed to be face centered cubic phases. The precursor impurities are not observed in XRD pattern. The crystalline size of the magnetic nanoparticles was determined from the Debye–Scherrer formula $D_{hkl} = 0.89\lambda/\beta \cos \theta$, where D is the crystallite size in nm, β is the full width at half maximum intensity in radian, θ is the Bragg diffraction angle and λ is the X-ray wavelength in nm [28]. The crystallite size of the $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs by using the Debye–Scherrer equation was found to be 20 nm. The ceria peak is not seen in XRD spectrum due to low percentage doping of ceria on the surface of Fe_3O_4 nanoparticles.

To confirm ceria present on Fe_3O_4 NPs, the SEM–EDX analysis was done. The SEM–EDX shows that 6% ceria is present on the surface of Fe_3O_4 NPs. Elemental mapping analysis shows that Ce, Fe and Oxygen elements co-exist in the nanoparticles and ceria is present on the surface of Fe_3O_4 NPs. Elemental mapping confirms that Ce, Fe, and O elements are uniformly distributed in $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs, which is shown in Fig. 3a–c.

The chemical status of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs was analyzed by X-ray photoelectron spectroscopy (XPS). The binding

Fig. 1 FTIR spectra of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs

Fig. 2 XRD pattern of the synthesized $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs

Fig. 3 SEM-EDX and elemental mapping showing uniform distribution of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs. **a** Oxygen distribution, **b** Fe distribution, **c** Ce distribution

energy can be used to determine the oxidation state. Therefore we can say that with an increase in a valence state of the element, its binding energy also increases. XPS survey spectra in Fig. 4a shows that the nanoparticles contained Ce, Fe, O and C [18, 29]. The Fe 2p XPS spectrum in Fig. 4b shows that the peaks at binding energies (BE) of 710 and 723 eV were of Fe^{2+} , while the peak at binding energies (BE) of 713 and 726 eV was characteristic of Fe^{3+} . The combination of Fe^{2+} and Fe^{3+} is confirmed due to satellite signal at 718 eV. Fe^{3+} and Fe^{2+} existing in the sample confirm the formation of Fe_3O_4 ,

whereas satellite signals at 715 eV and 719 eV are of Fe^{2+} and Fe^{3+} [29–31].

The lattice oxygen (O 1s) core level spectrum peak at 529 eV of Fe_3O_4 NPs is depicted in Fig. 4a [29, 32–35]. The XPS peak of Ce 3d doublet, its satellite peaks (S1 and S2), main Ce 3d_{5/2} and Ce 3d_{3/2} lines are shown in the Fig. 4c. The Ce two phases are identified by different positions and intensities of the shake up satellite peaks. The shape of measured Ce 3d line shows two satellite peaks. Its first satellite S1 is at 890 eV and the second satellite S2 is more intense at 897 eV. The satellite S2 partially overlaps with

Fig. 4 **a** XPS survey spectra for $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs, **b** XPS spectra for Fe in $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs, **c** XPS spectra for Ce in $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs

$3d_{3/2}$ line. The peak in range 915–920 eV is due to the oxygen vacancy in metal oxides that shows Ce^{3+} on the surface. However, the main Ce $3d_{5/2}$ line is seen at 882 eV, while Ce $3d_{3/2}$ is observed at 901 eV. The positions of peaks are in good agreement with the literature, which further supports the suggested phase identification [29, 32].

The Ce $3d$ binding energy is higher by 0.6 than reported literature, and Fe $2p$ binding energy is 0.5 eV less than the reported literature of Fe_3O_4 binding energy. These

Fig. 5 TEM analysis of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs

difference in binding energy is due to formation of CeO_2 composition on the surface of Fe_3O_4 [33, 34].

XRD and XPS both are surface analysis techniques as well as XPS is more sensitive than XRD. XPS detects signals within upper than 5 nm thickness and XRD detects signals in micro-order thickness on surface [35, 36]. To confirm the particle size of MNPs TEM analysis was carried out that shown in Fig. 5. The Particle size is found to be 9 nm and morphology of nanoparticles are found to be spherical in shape. XRD results show the crystallite size of 20 nm calculated by Scherrer formulae, whereas TEM analysis shows particle size of 9 nm.

3.2 Oxidase like activity: proof for the concept for $\text{CeO}_2\text{-Fe}_3\text{O}_4$ based detection

The oxidase like activity of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs is due to $\text{Ce}^{4+}/\text{Ce}^{3+}$ oxidation state and high mobility of surface oxygen. We have studied this activity by catalytic oxidation of catechol. The study explains the oxidation of catechol by $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs and the analogous absorption spectra for quinone complex generated by 9 nm particle size of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs. The color of particle dispersed catechol solution changed from transparent to brown. The intensity of the color is dependent on the concentration of catechol and $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs. These color changes are due to oxidation of catechol by $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs and formation of quinone complex, the schematic of mechanism is shown in Fig. 6. Oxidation and formation of quinone complexes with inner transition metals and phenolic compound by FTIR, XPS, and UV-vis spectroscopy have been reported previously [7].

XPS analysis (Fig. 4) shows that significant change in optical properties is due to Ce^{3+} and Ce^{4+} in $\text{CeO}_2\text{-Fe}_3\text{O}_4$ nanoparticle system. Therefore, catechol ligand forms quinone complex due to surface modification of

Fig. 6 Schematic mechanism of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs for catechol detection

$\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs. Quinone complex has a metal–catecholate bond with both sigma (σ) and π -donor bonding contribution [37]. Also, due to charge transfer, the colorless catechol gives colored quinone complex.

To confirm the oxidase-like activity of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs for catechol detection it was compared with tyrosinase enzyme. Tyrosinase enzyme changes optical properties of the reaction mixture and similar change in optical properties of the reaction mixture are shown by $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs. However, these nanoparticles can be used to replace conventional tyrosinase enzyme for catechol detection and exceptional method for polyphenol compounds detection.

3.3 Effect of catechol concentration

To study the effect of catechol concentration on tyrosinase enzyme and $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs, 1–7 mM catechol solution was prepared in pH 7.8 phosphate buffer solution (0.2 M). These catechol solutions were added to 5 mg of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs and 2 mL of 2000 ppm of tyrosinase (1 KU/mg) separately. Figure 7a, b show absorbance in the visible region by tyrosinase enzyme and $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs respectively. It can be inferred from the Fig. 7a, b that the colorless catechol solution gives colored quinone complex. Figure 7a, b demonstrates that with an increase in the catechol concentration, absorbance also increases and 5 mM catechol solution gives maximum absorbance for quinone complex. Hence, further experiments were carried out by using a 5 mM catechol solution. Figure 7c shows the calibration curve for catechol, where the absorbance of catechol increases linearly with the increasing concentration of catechol. The rapid color change and strong absorbance prove that $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs can be used for oxidation and as a color indicator reagent similar as tyrosinase for catechol detection.

Fig. 7 **a** Effect of catechol concentration by using tyrosinase enzyme, **b** effect of catechol concentration by using $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs, **c** calibration curve for catechol

3.4 Effect of ionic strength and buffer composition

Enzyme mimic properties of nanoparticles are greatly dependent on the buffer anions of reaction medium [10, 37]. Therefore, it is important to study the effect of ionic strength. Figure 8a shows that in the absence of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs the solution containing catechol in phosphate buffer is colorless and has no absorption in 400–800 nm. However, in the presence of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs, the solution becomes colored with maximum absorbance in a visible range (Fig. 8b). The absorbance originates from

Fig. 8 **a** UV-vis spectra in Buffer for (i) catechol (5 mM), (ii) $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs (5 mg), (iii) catechol (5 mM) with 5 mg $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs, **b** the color changed by $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs dispersed in catechol solu-

tion, **c** effect of buffer ionic strength on $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs interaction with 5 mM catechol, **d** effect of buffer ionic strength on particle size and zeta potential of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs

Table 1 Study of ionic strength on catechol oxidase activity of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs by using dynamic light scattering (DLS) and zeta potential

Sr. no.	Phosphate buffer concentration (M)	DLS particle size distribution (nm)	Zeta potential (mV)
1	0.05	406	-20.97
2	0.1	426	-20.83
3	0.2	555.4	-16.4
4	0.4	590.6	-10.85
5	0.6	864.1	-10.30
6	1	1442	-7.70

oxidation of catechol to colored quinone complex, similar to the phenomenon observed for tyrosinase enzyme in Fig. 8a [1]. To study ionic strength behavior, catechol oxidase like activity of nanoparticles in phosphate buffer solution (PBS) from 0.05 to 1 M at pH 7.8 was prepared. However, Fig. 8c shows 0.2 M phosphate buffer gives maximum absorbance. Higher concentration of PBS induced significant particle aggregation and was not used for detection. The occurrence of phosphate anion has an undesirable change in oxidase activity of nanoparticles. The decrease in absorbance was observed with an increase in ionic strength from 0.2 to 1 M buffer solution. The loss of reactivity in these buffers is due to the formation of metal phosphate on the surface of particles that results in partial inactivation of the surface. Therefore, they block the interconversion between $\text{Ce}^{4+}/\text{Ce}^{3+}$ and inhibit reaction with catechol.

To prove this assumption, dynamic light scattering (DLS) and zeta potential experiments were used to study the formation of metal phosphate and aggregation of particles. Figure 8d and Table 1 explains the values observed by increasing the concentration of PBS (0.05–1 M), which shows particle size increase from 406 ± 50 nm and change of zeta potential from -20.97 ± 2 to -7.70 ± 2 mV.

Change in surface properties of nanoparticles is observed by the change in zeta potential values, which confirms the formation of metal phosphate on the surface [1, 10]. DLS and zeta potential experimental results are given in Table 1. This shows that the change in the reaction is due to physicochemical properties of NPs as well as the buffer environment.

3.5 Effect of nanoparticle concentration

Reactive surface of the $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs are responsible for oxidase like activity and more the reactive surface of NPs more will be the activity. The different concentrations of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs ranging from 1 to 9 mg were added

Fig. 9 Effect of different concentrations of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs in pH-7.8 phosphate buffer (0.2 M) in presence of 5 mM catechol

to a fixed concentration of catechol and absorbance was measured by UV-vis spectrophotometer.

Figure 9 shows $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs in pH-7.8 phosphate buffer (0.2 M) in presence of 5 mM catechol. It can be concluded that absorbance change in different concentrations of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs was due to different chemical structures and redox potentials. It can be seen that 5 mg $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs shows maximum absorbance and this was selected as an optimum concentration for quinone complex formation [8, 9].

3.6 Effect of time

The inset of Fig. 10 shows the time-dependent catalytic activity of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs under standard conditions. In order to study the effect of time, 5 mg of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs was added in 5 mM catechol solution having phosphate buffer (0.2 M) of pH 7.8. The colorless catechol solution gives colored quinone complex, which shows absorbance

Fig. 10 Effect of time on catechol detection

in a visible region. Optical properties of the samples were measured at 2–20 min. As the reaction proceeds, the colorless solution becomes colored quinone complex, and maximum absorbance was observed at 14 min. Further increase in reaction time shows an insignificant change in the quinone complex formation. The difference in absorbance intensity is attributed by different catalytic activities of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs with time. Also, $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs with different physical properties have different chemical reactivities [8]. Therefore, 14 min was selected as the optimum time for $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs as enzyme mimic to detect catechol.

3.7 Reusability

A recyclable catalyst is key in the case of economic feasibility. $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs was successively reused until the fourth cycle (Fig. 11). After each cycle, $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs was washed with DI water three times and reused for the fresh cycle. After four consecutive cycles of reuse, it retains 82% residual activity and shows excellent reusability and durability of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs. Its activity might be reduced due to substrate poisoning [1].

3.8 Kinetics study

The oxidase like activity was further carried out by using 5 mg $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs in 5 mM catechol, prepared in phosphate buffer (pH-7.8, 0.2 M) up to 1 h. By using the equation below catechol concentration (Ca) was determined. Figure 12 illustrates that the concentration of the reactant (Ca) decreased linearly as time passed. This indicates that the reaction followed first-order kinetics with respect to the concentration of catechol.

$$Ca = Ca_0 + Cb_0 - Cb$$

Fig. 11 Reusability of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs

Fig. 12 Kinetics study of reaction

where Ca, catechol concentration at a time; Cb, quinone complex concentration at a time; Ca_0 , initial concentration of catechol; Cb_0 , initial concentration of quinone complex.

3.9 Interference study

It is essential to prevent the interference of ascorbic acid for the selective detection of catechol [38]. The study was carried out by adding ascorbic acid in pH 7.8 phosphate buffer containing $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs and catechol, the absorbance of colored solution was measured in the visible region and compared with the colorless ascorbic acid solution. The ascorbic acid solution does not change its color by adding $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs. Therefore from Fig. 13, it is concluded that there is no interference of ascorbic acid for catechol detection.

Fig. 13 Interference study of ascorbic acid for catechol detection by $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs

4 Conclusion

The current study explains the various effects of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ nanomaterials as enzyme mimic. The $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs have been used for the first time as enzyme mimic properties for catechol detection. The XPS study of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs reveals that the Ce, Fe and O Elements are present and the combination of Fe^{2+} , Fe^{3+} is confirmed due to the satellite signal. The colorless catechol to colored quinone complex follows first order kinetics. This is mostly due to the surface modification and charge transfer properties of nanoparticles. The quinone complex has the metal–catecholate bond with both sigma (σ) and pi (π) donor bonding contribution. On the other hand, DLS and zeta potential study shows that with an increase in ionic strength of PBS, oxidase like activity of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs decreases. This was due to the variation in surface properties of nanoparticles and was indicative of the formation of metal phosphate. Also, reusability of $\text{CeO}_2\text{-Fe}_3\text{O}_4$ NPs was studied till the fourth cycle in batch mode. The future study requires to deposit other metals on the Fe_3O_4 surface to know more about surface charges and oxidase mimic activity of metal nanoparticles. Such studies on enzyme mimicking nanoparticles help as guiding principle for the development of other nanoparticles based enzyme mimetics.

Acknowledgements The authors would like to acknowledge the University Grants Commission (UGC) of India for financial assistance in the research work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Hayat A, Cunningham J, Bulbul G, Andreescu S (2015) Evaluation of the oxidase like activity of nanoceria and its application in colorimetric assays. *Anal Chim Acta* 885:140–147. <https://doi.org/10.1016/j.aca.2015.04.052>
- Wei H, Wang E (2008) Fe_3O_4 magnetic nanoparticles as peroxidase mimetics and their applications in H_2O_2 and glucose detection. *Anal Chem* 80:2250–2254. <https://doi.org/10.1021/ac702203f>
- Mu J, Wang Y, Zhao M, Zhang L (2012) Intrinsic peroxidase-like activity and catalase-like activity of Co_3O_4 nanoparticles. *Chem Commun* 48:2540–2542. <https://doi.org/10.1039/c2cc17013b>
- Wei H, Wang E (2013) Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes. *Chem Soc Rev* 42:6060–6093. <https://doi.org/10.1039/c3cs35486e>
- Chaudhari KN, Chaudhari NK, Yu J-S (2012) Peroxidase mimic activity of hematite iron oxides ($\alpha\text{-Fe}_2\text{O}_3$) with different nanostructures. *Catal Sci Technol* 2:119–124. <https://doi.org/10.1039/c1cy00124h>
- Fiammengio R, Jäschke A (2005) Nucleic acid enzymes. *Curr Opin Biotechnol* 16:614–621. <https://doi.org/10.1016/j.copbio.2005.10.006>
- Hayat A, Andreescu D, Bulbul G, Andreescu S (2014) Redox reactivity of cerium oxide nanoparticles against dopamine. *J Colloid Interface Sci* 418:240–245. <https://doi.org/10.1016/j.jcis.2013.12.007>
- Peng X, Manna L, Yang W et al (2000) Shape control of CdSe nanocrystals. *Nature* 404:59–61. <https://doi.org/10.1038/35003535>
- Fang B, Kim M-S, Kim JH et al (2011) High Pt loading on functionalized multiwall carbon nanotubes as a highly efficient cathode electrocatalyst for proton exchange membrane fuel cells. *J Mater Chem* 21:8066. <https://doi.org/10.1039/c1jm10847f>
- Xue Y, Zhai Y, Zhou K et al (2012) The vital role of buffer anions in the antioxidant activity of CeO_2 nanoparticles. *Chem A Eur J* 18:11115–11122. <https://doi.org/10.1002/chem.201200983>
- Singh S, Dosani T, Karakoti AS et al (2011) A phosphate-dependent shift in redox state of cerium oxide nanoparticles and its effects on catalytic properties. *Biomaterials* 32:6745–6753. <https://doi.org/10.1016/j.biomaterials.2011.05.073>
- Han MJ, Yoo KS, Chang JY, Ha TK (2000) 5-(β -Cyclodextrinylamino)-5-deoxy- α -D-ribose as models for nuclease, ligase, phosphatase, and phosphorylase. *Angew Chem Int Ed* 39:347–349. [https://doi.org/10.1002/\(SICI\)1521-3773\(20000117\)39:2%3C347:AID-ANIE347%3E3.0.CO;2-Q](https://doi.org/10.1002/(SICI)1521-3773(20000117)39:2%3C347:AID-ANIE347%3E3.0.CO;2-Q)
- Muche MS, Gobel MW (1996) Bis(guanidinium) alcohols as models of staphylococcal nuclease: substrate binding through ion pair complexes and fast phosphoryl transfer reactions. *Angew Chem Int Ed Engl* 11:26–29. <https://doi.org/10.1002/anie.199621261>
- Wang Z, Yang X, Yang J et al (2015) Peroxidase-like activity of mesoporous silica encapsulated Pt nanoparticle and its application in colorimetric immunoassay. *Anal Chim Acta* 862:53–63. <https://doi.org/10.1016/j.aca.2014.12.046>
- Wang C, Chen W, Chang H (2012) Enzyme mimics of Au/Ag nanoparticles for fluorescent detection of acetylcholine. *Anal Chem* 84:9706–9712. <https://doi.org/10.1021/ac300867s>
- Tang Z, Wu H, Zhang Y et al (2011) Enzyme-mimic activity of ferric nano-core residing in ferritin and its biosensing applications. *Anal Chem* 83:8611–8616. <https://doi.org/10.1021/ac202049q>
- Lin Y, Ren J, Qu X (2014) Nano-gold as artificial enzymes: hidden talents. *Adv Mater* 26:4200–4217. <https://doi.org/10.1002/adma.201400238>
- Wang Z, Chen M, Shu J, Li Y (2016) One-step solvothermal synthesis of $\text{Fe}_3\text{O}_4\text{@Cu@Cu}_2\text{O}$ nanocomposite as magnetically recyclable mimetic peroxidase. *J Alloys Compd* 682:432–440. <https://doi.org/10.1016/j.jallcom.2016.04.269>
- Li S, Liu X, Chai H, Huang Y (2018) Recent advances in the construction and analytical applications of metal-organic frameworks-based nanozymes. *Trends Anal Chem* 105:391–403. <https://doi.org/10.1016/j.trac.2018.06.001>
- Zhao J, Dong W, Zhang X, Chai H, Huang Y (2018) $\text{FeNPs@Co}_3\text{O}_4$ hollow nanocages hybrids as effective peroxidase mimics for glucose biosensing. *Sens Actuators B* 263:575–584. <https://doi.org/10.1016/j.snb.2018.02.151>
- Li S, Wang L, Zhang X, Chai H, Huang Y (2018) A Co, N co-doped hierarchically porous carbon hybrid as a highly efficient oxidase mimetic for glutathione detection. *Sens Actuators B* 264:312–319. <https://doi.org/10.1016/j.snb.2018.03.015>
- Zhang W, Ma D, Du J (2014) Prussian blue nanoparticles as peroxidase mimetics for sensitive colorimetric detection of hydrogen peroxide and glucose. *Talanta* 120:362–367. <https://doi.org/10.1016/j.talanta.2013.12.028>
- Asati MA, Santra DS, Kaittanis MC et al (2010) Oxidase activity of polymer-coated cerium oxide nanoparticles. *Angew Chem*

- 48:2308–2312. <https://doi.org/10.1002/anie.200805279>. *Oxidase*
24. Chen Z, Yin JJ, Zhou YT et al (2012) Dual enzyme-like activities of iron oxide nanoparticles and their implication for diminishing cytotoxicity. *ACS Nano* 6:4001–4012. <https://doi.org/10.1021/nn300291r>
25. Niphadkar SS, Vetel MD, Rathod VK (2014) Purification and characterization of polyphenol oxidase from waste potato peel by aqueous two phase extraction. *Prep Biochem Biotechnol* 6068:37–41. <https://doi.org/10.1080/10826068.2014.940970>
26. Sojitra UV, Nadar SS, Rathod VK (2016) A magnetic tri-enzyme nanobiocatalyst for fruit juice clarification. *Food Chem* 213:296–305. <https://doi.org/10.1016/j.foodchem.2016.06.074>
27. Nadar SS, Rathod VK (2016) Magnetic macromolecular cross linked enzyme aggregates (CLEAs) of glucoamylase. *Enzyme Microb Technol* 83:78–87. <https://doi.org/10.1016/j.enzmt.2015.10.009>
28. Sojitra UV, Nadar SS, Rathod VK (2016) Immobilization of pectinase onto chitosan magnetic nanoparticles by macromolecular cross-linker. *Carbohydr Polym* 157:677–685. <https://doi.org/10.1016/j.carbp.2016.10.018>
29. Gawande MB, Bonifácio VDB, Varma RS et al (2013) Magnetically recyclable magnetite–ceria (Nanocat–Fe–Ce) nanocatalyst—applications in multicomponent reactions under benign conditions. *Green Chem* 15:1226. <https://doi.org/10.1039/c3gc40375k>
30. Wang N, Zhu L, Wang D et al (2010) Sono-assisted preparation of highly-efficient peroxidase-like Fe_3O_4 magnetic nanoparticles for catalytic removal of organic pollutants with H_2O_2 . *Ultrason Sonochem* 17:526–533. <https://doi.org/10.1016/j.ultsonch.2009.11.001>
31. Gupta H, Paul P, Kumar N et al (2014) One pot synthesis of water-dispersible dehydroascorbic acid coated Fe_3O_4 nanoparticles under atmospheric air: blood cell compatibility and enhanced magnetic resonance imaging. *J Colloid Interface Sci* 430:221–228. <https://doi.org/10.1016/j.jcis.2014.05.043>
32. Jain R, Dubey A et al (2015) Gas–solid interaction of H_2 -Ce $_{0.95}\text{Zr}_{0.05}\text{O}_2$: new insights on surface participation in heterogeneous catalysis. *Catal Sci Technol*. <https://doi.org/10.1039/C5CY01428J>
33. Beche E, Charvin P, Perarnau D, Abanades S, Flament G (2008) Ce 3d XPS investigation of cerium oxides and mixed cerium oxide (Ce $_{x}\text{TiyO}_z$). *Surf Interface Anal* 40:264–267. <https://doi.org/10.1002/sia.2686>
34. Uhlig I, Szargam R, Nesbitt H, Laajalehto K (2001) Surface states and reactivity of pyrite and marcasite. *Appl Surf Sci* 179:222–229. [https://doi.org/10.1016/S0169-4332\(01\)00283-5](https://doi.org/10.1016/S0169-4332(01)00283-5)
35. Ai Z, Zhang L, Lee S, Ho W (2009) Interfacial hydrothermal synthesis of Cu@Cu $_{2}\text{O}$ core–shell microspheres with enhanced visible-light-driven photocatalytic activity. *J Phys Chem C* 113:20896–20902. <https://doi.org/10.1021/jp9083647>
36. Movahed SK, Dabiri M, Bazgir A (2014) A one-step method for preparation of Cu@Cu $_{2}\text{O}$ nanoparticles on reduced graphene oxide and their catalytic activities in N-arylation of N-heterocycles. *Appl Catal A Gen* 481:79–88. <https://doi.org/10.1016/j.apcata.2014.04.023>
37. Turcheniuk K, Tarasevych AV, Kukhar VP et al (2013) Recent advances in surface chemistry strategies for the fabrication of functional iron oxide based magnetic nanoparticles. *Nanoscale* 5:10729–10752. <https://doi.org/10.1039/c3nr04131j>
38. Rashid M, Auger V, Ali Z (2014) Simultaneous electrochemical detection of dopamine, catechol and ascorbic acid at a poly(acriflavine) modified electrode. In: XIII Mediterranean conference on medical and biological engineering and computing 2013. Springer, Basel. https://doi.org/10.1007/978-3-319-00846-2_221

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.