Green Synthesis of Cobalt and Iron Incorporated Citric Acid/β-cyclodextrin Composites: Efficient H_2O_2 Scavengers

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Abstract

Sonochemical procedure was used to synthesize Co/β-cyclodextrin complex. For the first time Fe/β-cyclodextrin complex was also synthesized in alkaline media. Moreover, β-cyclodextrin was modified by citric acid (CA) and reacted with metal salts under ultrasonic irradiation. The prepared catalysts were characterized by using EDX, UV-vis and FTIR analytical techniques. The highest incorporation of metal into β-cyclodextrin was observed in the citric acid cross linked β-CD. The catalase like activity (peroxide scavenging) of Co/β-CD and Fe/β-CD complexes and composites was evaluated by UV–visible spectrophotometer and their superoxide scavenging capacity were measured using the pyrogallol method. The Co_3/CA-β-CD complex showed the best catalase mimetic activity (93.2%) even in comparison to catalase enzyme extracted from potato. By contrast Fe_3/CA-βCD complex had stronger capability to eliminate superoxide anion radicals.

1 Introduction

Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, organic peroxides, and hydroxyl radical, are a consequence of aerobic metabolism [1–3]. In healthy systems, ROS are efficiently regulated by the defensive enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidases [4, 5]. Catalase and peroxidase enzymes are important antioxidant metalloenzymes that catalyze the decomposition of hydrogen peroxide into water, protecting cells from its toxic effects. Catalase contains four porphyrin heme groups that H_2O_2 degradation occurs at the iron atom of the porphyrin [6]. In humans, SOD is a dimeric-protein consists of copper(II) and zinc(II) ions bridged by a imidazolate anion that catalyzes the dismutation of superoxide radical (O_2^-) to H_2O_2 and O_2 [7].

Creating artificial enzymes that mimic the complexity and function of natural systems have been a great challenge for the past two decades [8–11]. To overcome intrinsic drawbacks of the practical application of enzymes (high cost, low stability against denaturation and deactivation, the difficulties in recovery and recycling), considerable attention is being paid to designing low molecular weight and low cost synthetic metal complexes such as SOD and/or catalase and peroxidase mimics [12].

On the other hand, in the last decades, β-cyclodextrin (β-CD) derivatives are used in pharmaceutical applications for numerous purposes, including improving the solubility, stability, safety and bioavailability of drugs [13, 14]. The external surface of β-CD is hydrophilic due to it containing hydroxyl groups, while its inner cavity is hydrophobic as it is lined with ether-like anomeric oxygen atoms. This cavity makes it such useful tool, as they are capable of including guest molecules of different types [15, 16]. Recently, β-cyclodextrin polymers crosslinked by citric acid (CA) that are synthesized without any organic solvent and harmful additive are of great research interest. The carboxyl (–COOH) groups in these polymers provide adsorption sites for metals [17, 18]. Citric acid has also exhibited antioxidant and anti-inflammation properties [19]. Furthermore, Wang et al. demonstrated Co_3O_4 nanoparticles exhibit intrinsic peroxidase- and catalase-like activity [20]. With this in mind, herein, we designed the iron and cobalt complexes and composites, derived from β-cyclodextrin, under ultrasound irradiation. The antioxidant activity of prepared

compounds are investigated; as such an activity is of pharmaceutical interest for protection against oxidative stress.

2 Experimental

2.1 Materials

β-cyclodextrin (98%) was purchased from S.D. Fine chemicals, Mumbai. $FeSO_4.9H_2O$ (99%), $CoSO_4.7H_2O$ (99.5%), Citric acid monohydrate ($C_6H_8O_7.H_2O$ 99.5%), H_2O_2 (30%) and Mohr's salt (≥ 99%) were purchased from Merck. 1,10-phenanthroline monohydrate (99%) was purchased from Sigma-Aldrich. They were used without further purification.

2.2 Apparatus and methods

Metal contents of obtained complexes were estimated using SEM-EDS (VEGA\\TESCAN-LMU, Czech Republic) spectrometer under vacuum mode for precise measurement of both light and heavy elements. FTIR spectra were recorded on a Bruker Tensor 27 instrument using KBr pressed powder discs. UV–Vis spectra were recoeded on a Shimadzu UV-1700 spectrophotometer and referenced against a solvent blank.

2.3 Sonochemical synthesis of Fe/β-cyclodextrin

β-cyclodextrin (0.44 g, 0.39 mmol) was dissolved in deionized water (25 mL). For deprotonation of β-CD, the pH of solution was set equal to 10 by dropwise addition of NaOH 0.25 M and the solution was allowed to stir for 1 h. The resulting sample was dried in the oven at 80 ºC.

In the second step, 0.33 g of $FeSO_4.9H_2O$ (1.17mmol) was dissolved in 25 mL deionized water, and added to deprotonated β-cyclodextrin (Fe/β-CD molar ratio of 3). The green mixture was sonicated for 1 hour at 60% amplitude at 37 ºC. After sonication to resultant solution was added about 300 mL of ethanol. The precipitate was then filtered, washed with water and air dried. The Fe/β-CD prepared in this ratio is designated in the next as Fe_3/β-CD.

2.4 Sonochemical synthesis of Co/β-cyclodextrin

In a typical synthesis [21], 1.16 g of β-CD (1 mmol) was dissolved in 50 mL NaOH 0.5 M,and slowly added to a stirring solution of $CoSO_4.7H_2O$ (75 ml 0.04 M; Co/β-CD molar ratio of 3). The complex formed immediately giving a strong green coloured solution. The reaction mixture was then irradiated with ultrasound for one hour (37ºC). At the end of the sonication process, the resulting solution was filtered to remove solid particles. To this dark-green solution was added about 300 mL of ethanol until a green-gray suspension was formed, which was filtered, and then air dried at room temperature. The Co/β-CD prepared in this ratio is designated in the next as Co_3/β-CD.

For comparison, Fe/β-cyclodextrin and Co/β-cyclodextrin complexes were also synthesized with a metal/β-CD molar ratio of 1. The materials prepared in this ratio are designated in the next as M_1/β-CD.

2.5 Preparation of Co/CA-βCD & Fe/CA-βCD

To prepare β-cyclodextrin (β-CD) crosslinked by citric acid, 0.68 g of citric acid (3.5 mmol) was dissolved in 5 mL water; 0.5 g of NaH_2PO_4 was added to the beaker. After dissolving of sodium dihydrogen phosphate, (1.75 mmol) β-CD was mixed in beaker and the resulting solution was placed in an ultrasonic apparatus for sonication to obtain uniform solution. The reaction mixture was refluxed at 100°C for 3 h, and ethanol was added to precipitate the product. The precipitant was centrifuged and dried at 60°C.

0.71 g of composite was added to 25 mL of $CoSO_4.7H_2O$ 4mM solution (1:2) and sonicated for one hour. Then the supernatant was removed, added to ethanol and dried at ambient temperature.

Fe/CA-βCD was also synthesized with the same condition using 44 mL of $FeSO_4.9H_2O$ 4mM solution.

2.6 Hydrogen peroxide scavenging assay

1,10-phenanthroline and ferrous ammonium sulphate were used to detect H_2O_2 scavenging activity of anti-oxidants. To a test tube, 0.01 g complex and 1 mL of hydrogen peroxide (at a concentration of 10 mM) were added there after incubated at room temperature. After incubation, 1 mL of ferrous ammonium sulphate (1 mM) and 3 mL of 1 mM 1,10-phenanthroline were added to test tube, mixed well and incubated for 30 minutes at room temperature. Finally absorbance was taken at 510 nm through a spectrophotometer. The blank solution contained only ferrous ammonium sulphate (1 mL, 1mM) and 1,10-phenanthroline (3 mL, 1 mM) and showed maximum absorbance. The ability of compounds having hydrogen peroxide scavenging activity was calculated using following formula:

$$\%\ H_2O_2 \text{ scavenging activity} = A_{test} / A_{blank} \times 100$$

where A_{test} is the absorbance of the solution containing ferrous ammonium sulphate, 1,10-phenanthroline, hydrogen peroxide along with prepared complex.

For comparison, catalase enzyme extracted from potato was also evaluated by this method. An extract of potato was prepared by chopping it up into tiny pieces, placing the container on crushed ice and removing large chunks. The potato extract contains 100 units of catalase/ml.

The Catalase activity measurement was modified for potato extract to be able to record UV-vis spectra; 1 g of potato extract containing catalase was placed in test tube and 1 mL of hydrogen peroxide (1 mM),1 mL of ferrous ammonium sulphate (1 mM) and 3 mL of 1 mM 1,10-phenanthroline were added to test tube.

2.7 Superoxide scavenging capacity

The scavenging ability on superoxide anion radical was determined by the indirect method of pyrogallol autoxidation. To 0.01 g of the complex, 3 mL of Tris–HCl buffer (50 mM, pH 8.2) was added. The mixed solution was stirred and then pyrogallol (1 mL, 9mM) was added to reaction mixtures. An increase in absorbance is recorded at 320 nm for 3 min by spectrophotometer [22]. The scavenging ability was calculated by the following formula:

Scavenging ability (%) = $(1- A_2/A_1) \times 100$, where

A_1 = Absorbance change due to pyrogallol autoxidation in the system control (without Catalyst)

A_2 = Absorbance change due to pyrogallol autoxidation in the sample reaction

3 Results & Discussion

Chelating properties of β-CD can be enhanced in alkaline solutions due to the deprotonation of hydroxyl groups. Matsui and co-workers reported the possible formation of 1:1 dihydroxy – Cu(II)–β-CD complex in alkaline solution (pH = 12) in which β-CD was in the form of double deprotonated anion. In turn, formation of solely dinuclear Cu_2-β-CD complex without hydroxy bridges was observed in alkaline aqueous medium (pH = 12.5) under conditions of metal ion excess [23, 24]. Interactions of other metal ions with unmodified cyclodextrins have been studied to a lesser extent. On the other hand, from a solution of Fe salt in water at pH = 12, a precipitate of $Fe(OH)_3$ is formed and the deprotonated β-CD does not interact with Fe (III) ions. Therefore, a proper concentration of NaOH is essential to the formation of Fe/β-CD. We presented the synthesis of Fe/β-CD via a green approach in two step.

3.1 UV-Vis spectroscopy

The UV-visible spectra of cobalt and iron complexes are plotted together in Fig. 1. In the visible region, the absorption spectra of the aqueous solutions of Co/CA-βCD exhibit a broad band in the range of 500–600 nm, of assignable to low intensity d-d band.

The citric acid containing complexes also exhibited a weak absorption band at 280 nm that was likely to be oxygen-to-metal charge transfer transition. The presence of LMCT bands confirmed that cobalt and iron were coordinated to the β-cyclodextrin. Furthermore, the intensity of the spectra significantly increased with increasing in the metal : β-CD ratio.

3.2 FT-IR spectroscopy

The characteristic peaks of β-CD were observed at 1461 cm^{-1}, 1155 cm^{-1} and 1032 cm^{-1}, corresponding to CH_2 bending, C-O-C asymmetric glycosidic vibration and C-O stretching vibration, respectively (Fig. 2). Thus it is assumed that its structure remains intact upon complexing. The absorption bands in the region 550–800 cm^{-1} belong to the deformation vibrations of the C-H bonds and the pulsation vibrations in glucopyranose cycle. In addition, three peaks at 3413 cm^{-1}, 2923 cm^{-1} and 1635 cm^{-1} were indicative of

the O-H stretching vibration, C-H asymmetric stretching and H-O-H bending vibration. A sharp peak at 3740 cm^{-1} accompanied by the broad band in the range 3600 – 3000 cm^{-1} belongs to the non-hydrogen bonded OH groups of β-CD.

Figure 3 shows the FT-IR spectra of CA-βCD and Co/CA-βCD. The composites exhibited an additional band of 1730 cm^{-1} with that of β-cyclodextrin. The new band can be attributed to stretching vibration of carboxyl ester groups, which showed the esterification reaction between carboxyl groups on citric acid and hydroxyl groups on β-cyclodextrin was successfully carried out and confirmed the formation of ester groups

3.3 Chemical composition analysis using EDX

EDX elemental analysis was used to estimate the quantity of metals in the catalysts. Figure 4 shows the representative EDX spectra of prepared samples. Data showed the higher metal content in complexes with a 3:1 metal/C molar ratio in comparison to complexes with a 1:1 metal/C molar ratio. The results suggested that iron was present, at appreciable levels and the two step procedure was excellent to produce Fe/β-CD. Furthermore the absence of NaOH traces on the surface of Fe_3/β-CD in spite of the presence of Na ions in other complexes, approved the complete replacement of Na^+ with Fe^{3+} (see Supplementary information). Apparently iron was loaded to β-cyclodextrin more than cobalt (Table 1). Furthermore, the addition of citric acid to the β-cyclodextrin enhanced metal loading, significantly.

Table 1
The metal content of β–CD complexes determined by EDX analysis

	M_1/β-CD	M_3/β-CD	M_1/CA-β-CD	M_3/CA-β-CD
Cobal (wt%)	3.6	16.8	15.6	30.0
Iron (wt%)	3.9	19.4	12.2	34.8

3.4 SEM analysis

The surface features of the CA-βCD, Co_3/CA-βCD and Fe_3/CA-βCD are provided in Fig. 4. CA-βCD was revealed as a sheet-like structure that were irregular in shape. The original morphology of both Co_3/CA-βCD and Fe_3/CA-βCD were preserved and partial agglomeration of metals was observed.

3.5 Hydrogen peroxide scavenging activity

The ability of the prepared complexes to scavenge hydrogen peroxide was measured using an iron(II)-phenanthroline complex. This assay is based on the reaction of ferrous ion (Fe^{+2}) with 1,10-phenanthroline. Ferrous ion specifically forms red-orange tri-phenanthroline complex which absorbs maximally at 508–510 nm. It is known that if hydrogen peroxide is added to the tube before addition of 1,10-phenanthroline, then H_2O_2 will oxidize all the ferrous ion to ferric ion which is incapable of forming red-orange complex with 1,10-phenanthroline and a sharp reduction in A_{510} can be seen. If a scavenger is

added in the sample and followed by known amount of H_2O_2 for few minutes, no ferrous to ferric conversion will occur and detect by addition of 1,10-phenanthroline which yields a red-orange complex.

The results are shown in Fig. 4. The Co_3/CA-βCD complex exhibited the best performance among the studied catalysts for scavenging H_2O_2 (Co_3/CA-βCD > Co_3/βCD > Co_1/CA-βCD > Co_1/βCD ; 93.2 > 77.5 > 66.2 > 62.4). Moreover in cobalt complexes of β-CD the higher Coloading was associated with a better activity and in good agreement with the EDX results.

However by using citric acid, the complex with low cobalt content showed comparable activity to Co_3/βCD. It was found that citric acid as a natural antioxidant scavenges hydrogen peroxide [19].

On the contrary, catalase like activity decreased with the increase in iron content (Fe_3/CA-βCD > Fe_3/βCD > Fe_1/CA-βCD > Fe_1/βCD ; 21.9 > 27.0 > 53.9 > 71.2). It could be concluded that the cobalt ion remained in the divalent state, which was considered as an active catalyst for reaction with hydrogen peroxide (Fenton-like reaction) while the oxidation state of Fe (III) ions did not change. This indicates that the metal oxidation state plays a major role in its H_2O_2 scavenging ability activity.

Certain plants and animal organs contain high concentrations of catalase. Potatoes and liver are two commonly used sources of catalase. We prepared an extract of potato to compare its catalase-like activity with the prepared complexes of β-cyclodectrin.

The Catalase activity of 0.1 g of potato extract in the presence of H_2O_2 10 mM was very low. So different concentrations of potato extract and H_2O_2 were tested to aim a measurable amount; the results showed 1 g of potato extract and 1 mL of hydrogen peroxide (1 mM) had comparable activity to Co_3/β-CD (Fig. 5). For decomposition of H_2O_2 at concentration of 10 mM, much more potato extract was needed. It can be concluded that Co_3/β-CD had considerably higher activity compared to potato extract.

3.6 Superoxide scavenging capacity

The superoxide scavenging potential of prepared complexes was evaluated by measuring the inhibition of pyrogallol autoxidation that is catalyzed by the superoxide radical (scheme 1)[25].

Pyrogallol autoxidizes rapidly in aqueous solution and several intermediate products are apparently formed. Thus the solution first becomes yellow-brown with a spectrum showing a shoulder between 400 and 425 nm. After a number of minutes the colour begins to turn green and finally, after a few hours, a yellow colour appears. Generally in the UV-Vis spectra recorded during the autoxidation process, three absorption maxima evolved with time; at 270–275 nm, 310–320 nm and 420–440 nm [26]. In the present investigation, the autoxidation was taken from the linear increase in absorbance at 325 nm.

The results of the superoxide scavenging activity (Fig. 6) showed that all synthesized complexes were less effective in comparison to scavenging hydrogen peroxide. However the reaction rate increased proportionally with iron content and the Fe_3/CA-βCD complex displayed the highest catalytic performance

(Fe_3/CA-βCD > Fe $_3$/βCD > Fe $_1$/CA-βCD > Fe $_1$/βCD ; 37.2 > 31.39 > 23.49 > 17.1). It can be concluded that the synthesized complexes and composites are more suitable for trapping hydrogen peroxide than removing superoxide radical.

Moreover the capacity of the Co/β-CD complexes for superoxide scavenging was found to be considerably lower than that of the Fe/β-CD complexes and the catalytic activity of cobalt complexes was enhanced on decreasing the metal amount. Maybe it's because upon binding of a pyrogallol ligand to Fe^{3+}, the polyphenol can reduce the iron to Fe^{2+} [27], while the reduction of cobalt does not happen under normal circumstances.

4 Conclusions

This work was aimed at the production of cobalt and iron β-cyclodextrin with a simple, cost-efficient and green procedure. Citric acid-cross linked β-cyclodextrin supported cobalt and iron were also synthesized. We evaluated the potential of prepared complexes in H_2O_2 and superoxide radical scavenging. Co_3/CA-βCD demonstrated pronounced catalase mimetic activity (93.2%) and Fe_3/CA-βCD complex displayed the highest catalytic performance in superoxide removal. In conclusion cobalt based complexes of β-CD may be suitable and useful agents in biological systems, where they may act as synthetic protector molecules for hydrogen peroxide.

Declarations

Acknowledgment

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Figures

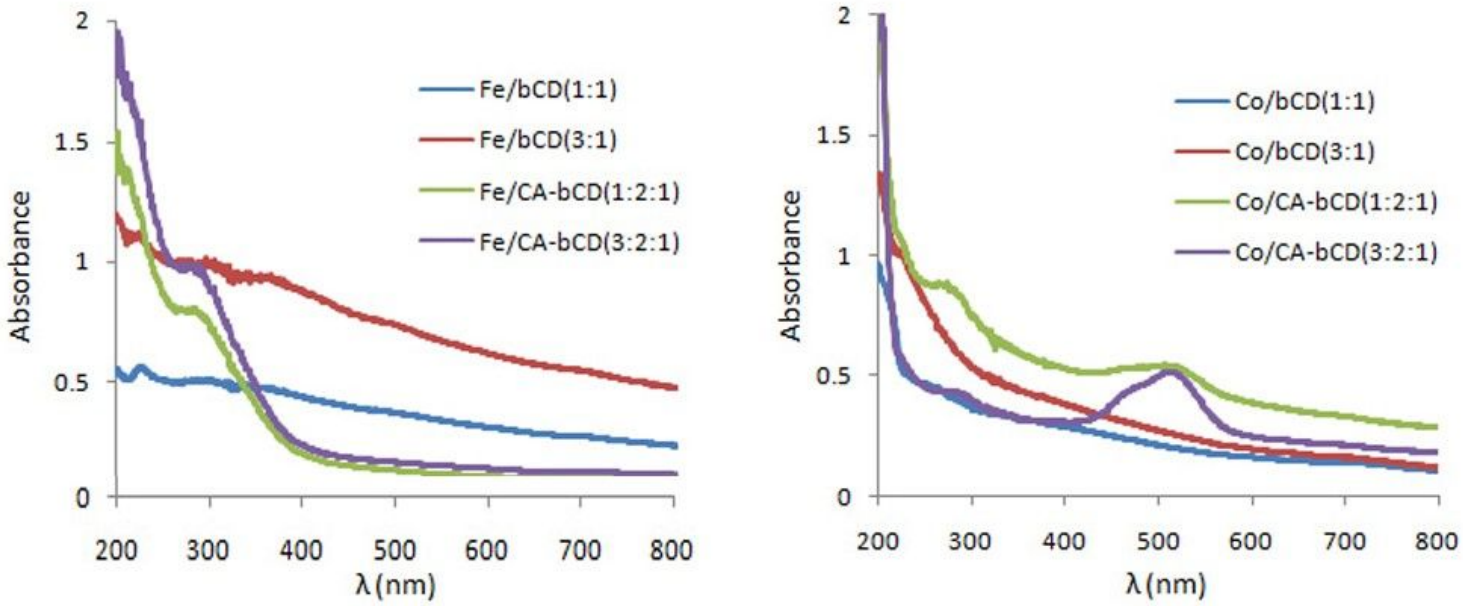

Figure 1

UV-vis spectra of Fe/β-CD (left) and Co/β-CD (right) complexes

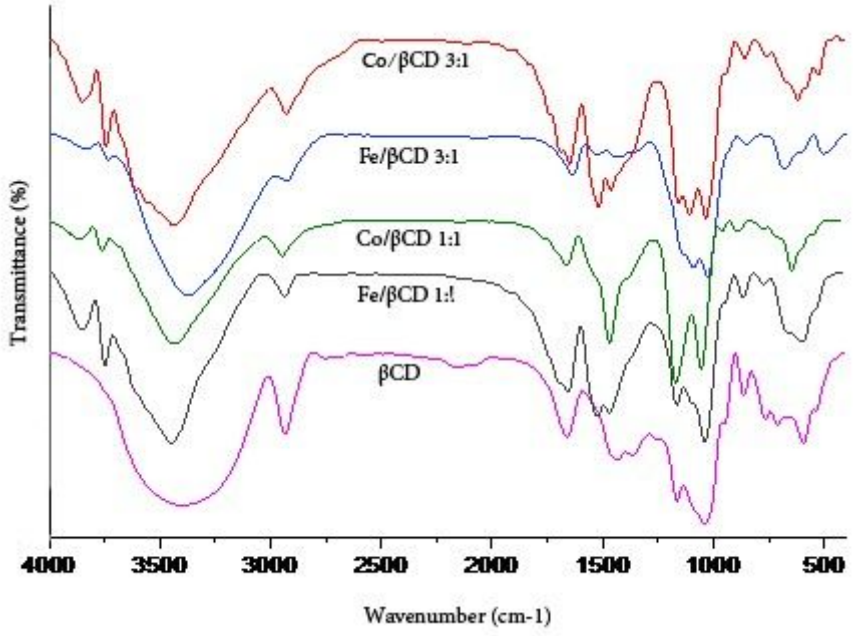

Figure 2

FT-IR spectra of β-CD, Co/β-CD and Fe/β-CD complexes

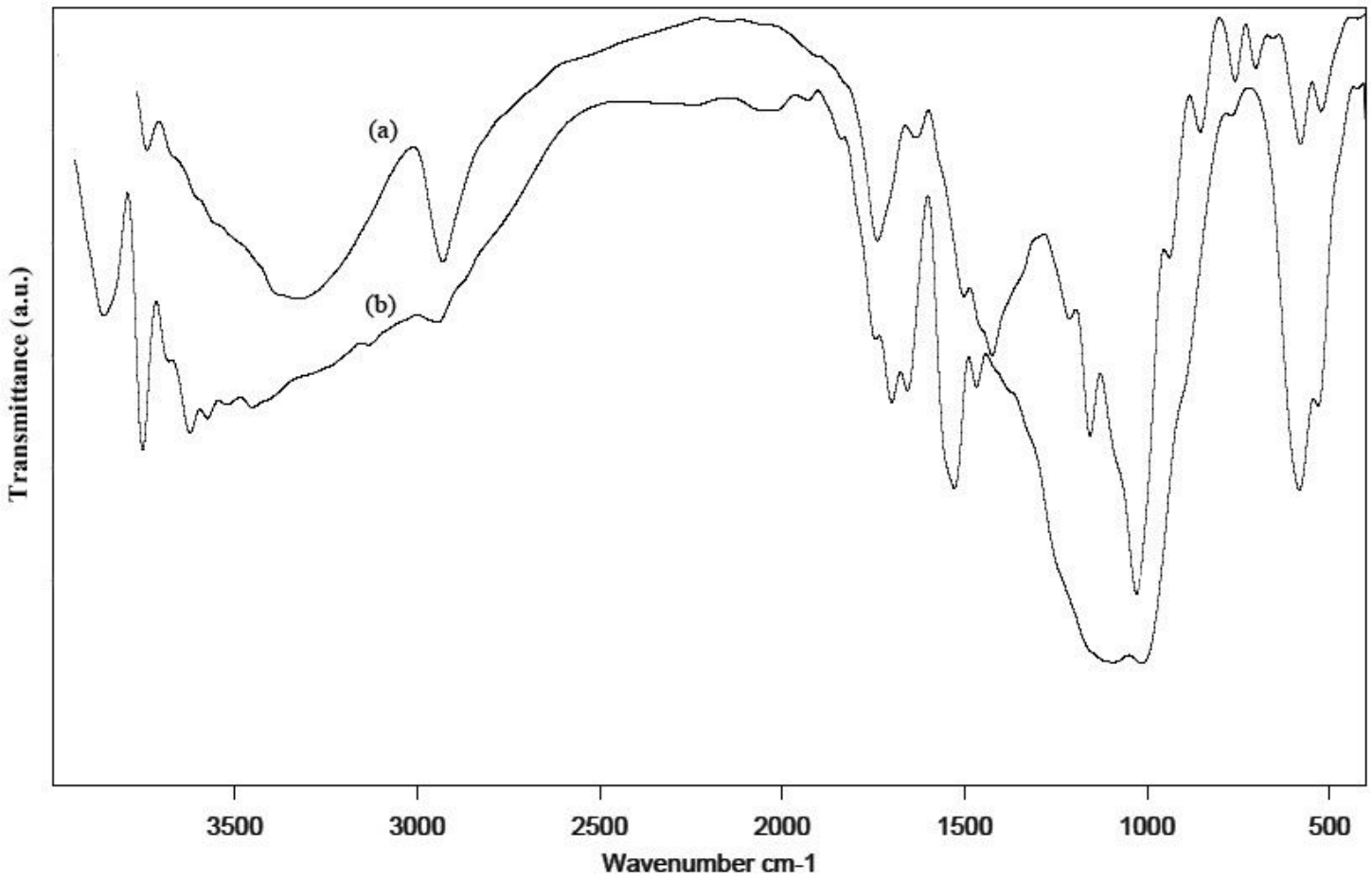

Figure 3

FT-IR spectra of (a) CA-βCD and (b) Co/CA-βCD composite

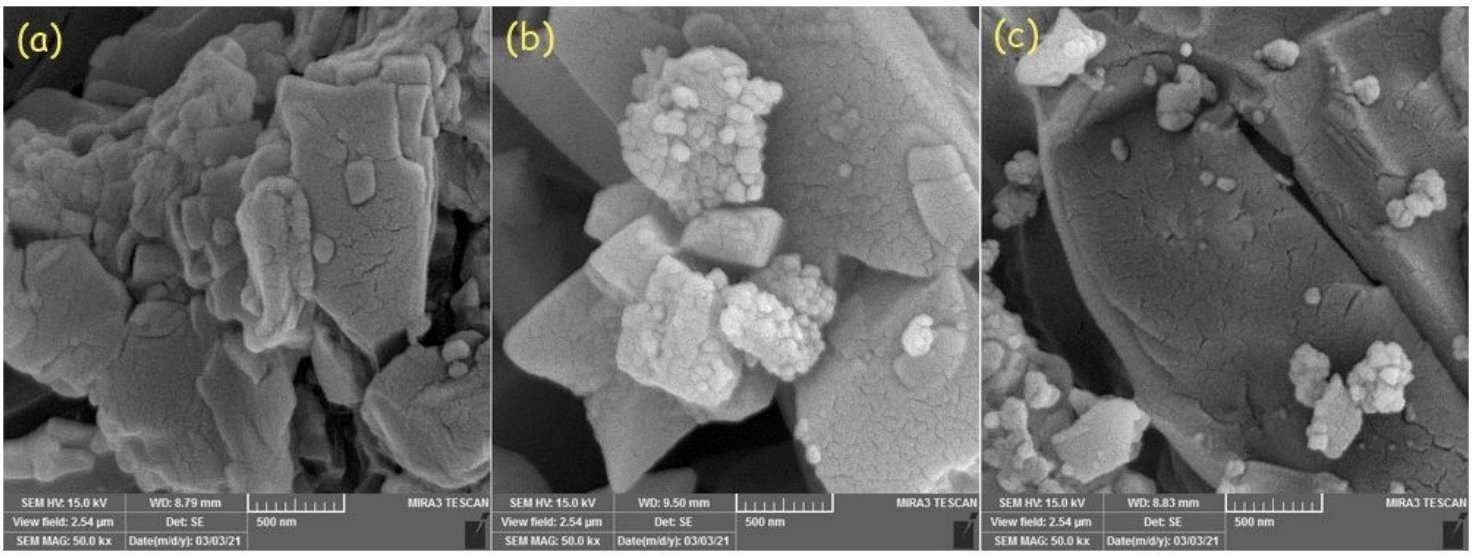

Figure 4

SEM images of a) CA-βCD b) Co3/CA-βCD c) Fe3/CA-βCD (×50kx)

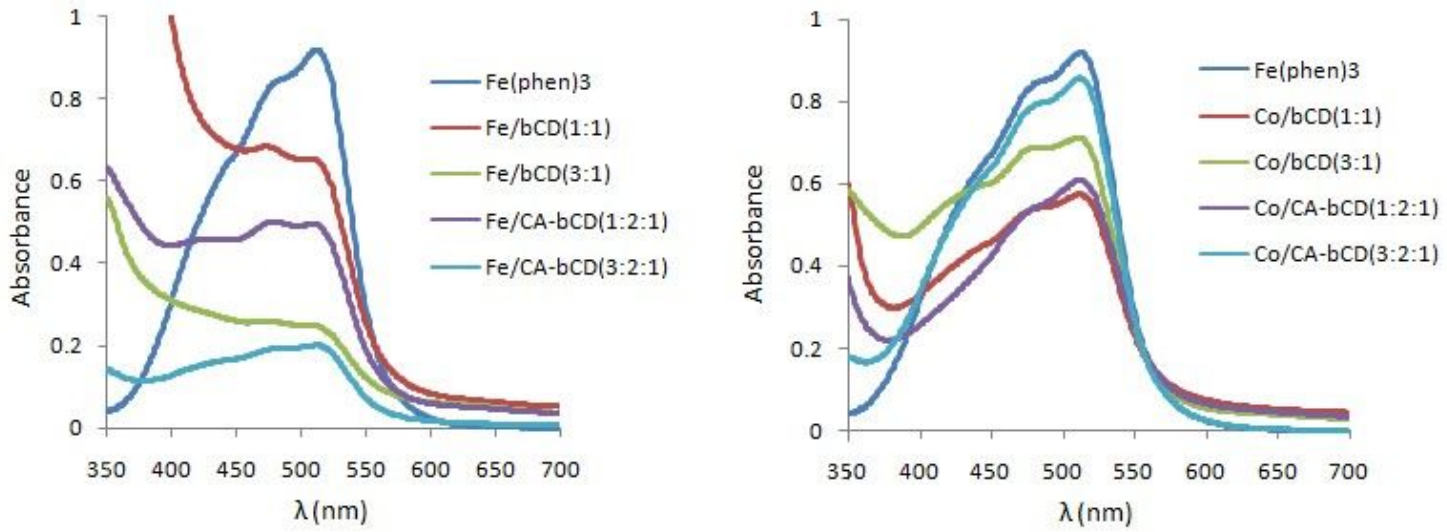

Figure 5

Catalase-like activity of cobalt and iron β-cyclodextrin complexes and composites

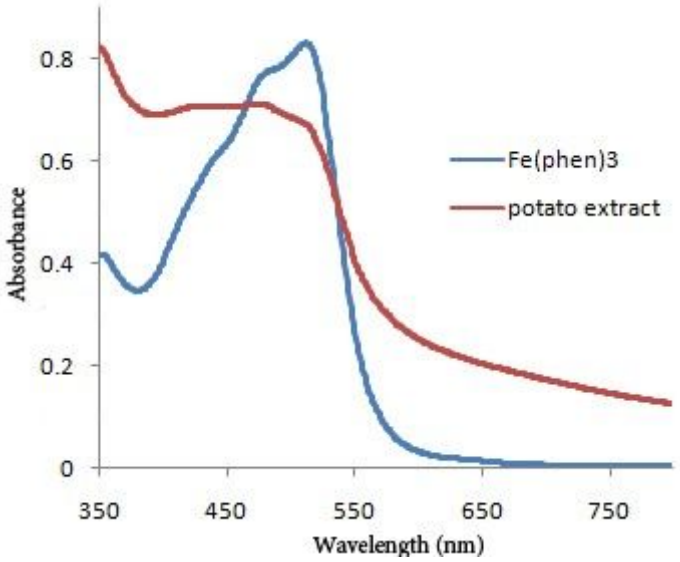

Figure 6

Catalase-like activity of potato extract

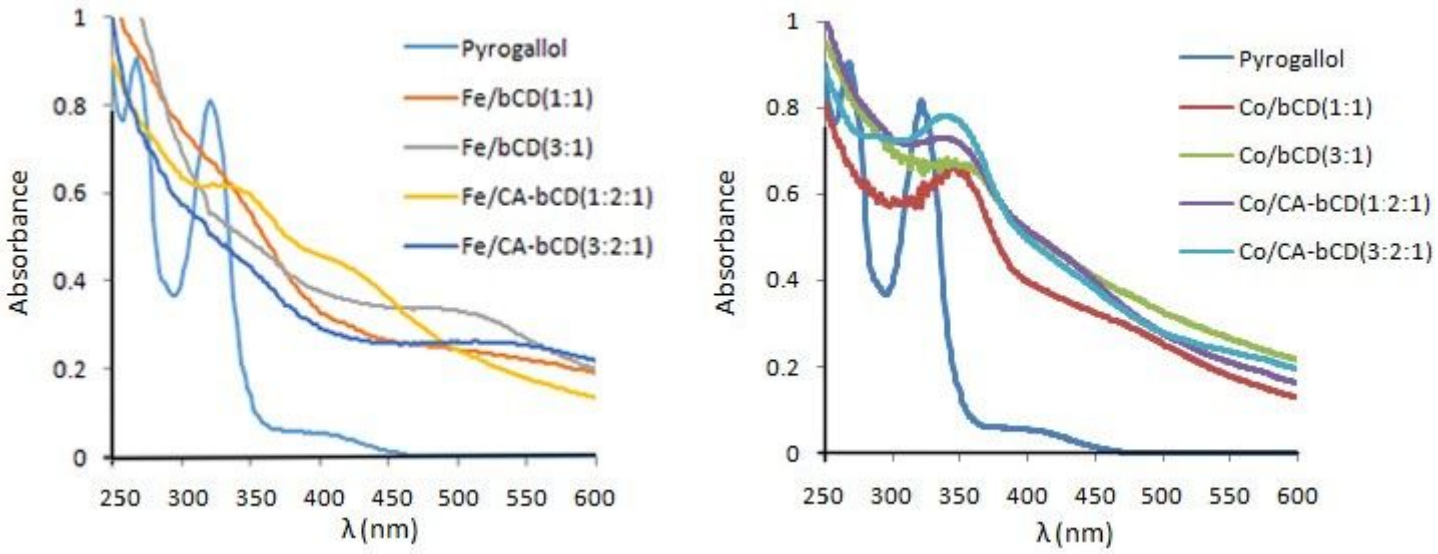

Figure 7

UV-Vis spectrum of pyrogallol autoxidation in the presence of prepared samples

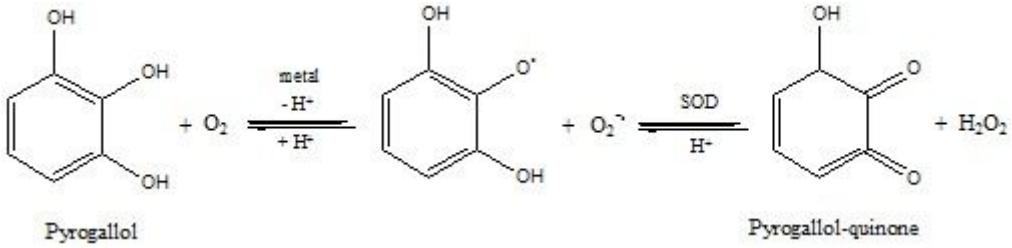

Figure 8

Pyrogallol autoxidation catalyzed by the superoxide radical

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