

Synthesis and antioxidant activity of biocompatible β -cyclodextrin-stabilized aqueous sols of nanocrystalline ceria



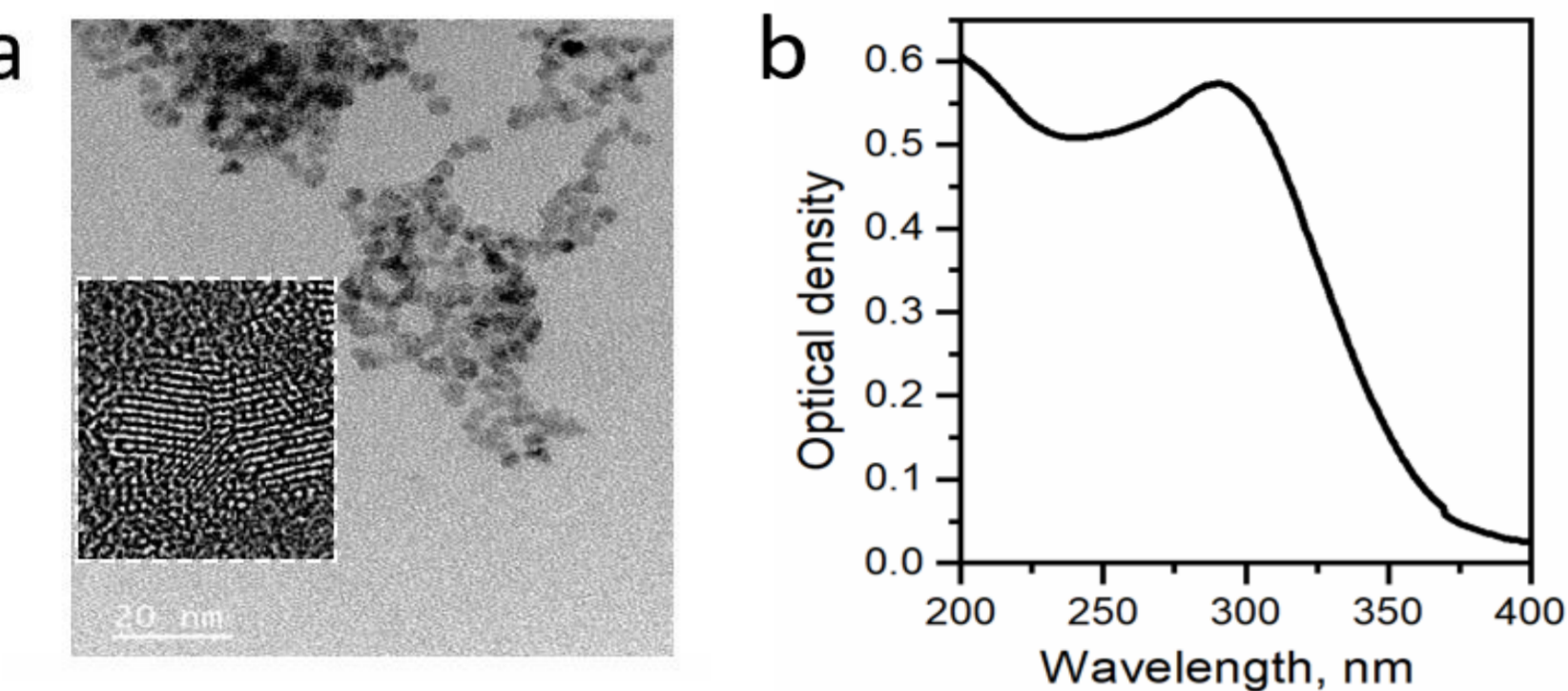
Grygorova G.V., Seminko V.V., Yefimova S.L.
Institute for Scintillation Materials of the NAS of Ukraine
60 Nauky ave., 61072-Kharkiv, Ukraine
E-mail: grigorova@isma.kharkov.ua



Introduction

Cerium oxide nanoparticles (nanoceria) are potent free-radical scavengers because of their unique antioxidant properties determined by switching of oxidation states between Ce^{3+} and Ce^{4+} in ambient conditions. To increase the antioxidant activity of CeO_2 , a variety of methods have been proposed, such as coating nanoceria with organics, doping nanoceria with metal ions, changing the buffer anion, adjusting the size and shape of nanoceria, and so on. On the other hand, β -cyclodextrin molecules have attracted great attention due to excellent biocompatibility, non-toxicity and capacity of these organic structures to include guest molecules within their hydrophobic cavities improving physicochemical properties of the guest. In this study, β -CD@ CeO_2 are synthesized via the simple co-precipitation method in alkaline media using unmodified β -cyclodextrin (β -CD) as a solubilizer and stabilizer agent. These NPs potentially can combine the strong antioxidant properties with ability to carry hydrophobic substances (including antioxidants, small drug molecules etc.) to various cell targets for improved therapy of various diseases.

Results and discussion



Transmission electron microscopy (TEM) analysis of β -CD@ CeO_2 NPs shows the presence of discrete nanocrystals with an average size of 5 nm (Figure 1a), while high-resolution TEM (HR-TEM) shows a crystalline nanoceria core (the insert in Fig. 1a). Through DLS measurements, the average intensity-weighted hydrodynamic diameter of NPs was determined to be 30.2 ± 1.4 nm with a PDI of 0.235. The larger hydrodynamic diameter of β -CD@ CeO_2 NPs in aqueous solution is likely attributed to a hydration layer surrounding β -CD@ CeO_2 . Fig. 1b shows the UV absorption spectrum of β -CD@ CeO_2 . The ceria nanoparticles exhibit a strong absorption below 400 nm with a well-defined absorbance peak at 292 nm assigned to the charge transfer between the O 2p and Ce 4f states of O^{2-} and Ce^{4+} .

Fig. 1. a) TEM image of β -CD@ CeO_2 NPs. Inset: HRTEM pattern; b) UV absorption spectrum of β -CD@ CeO_2 NPs

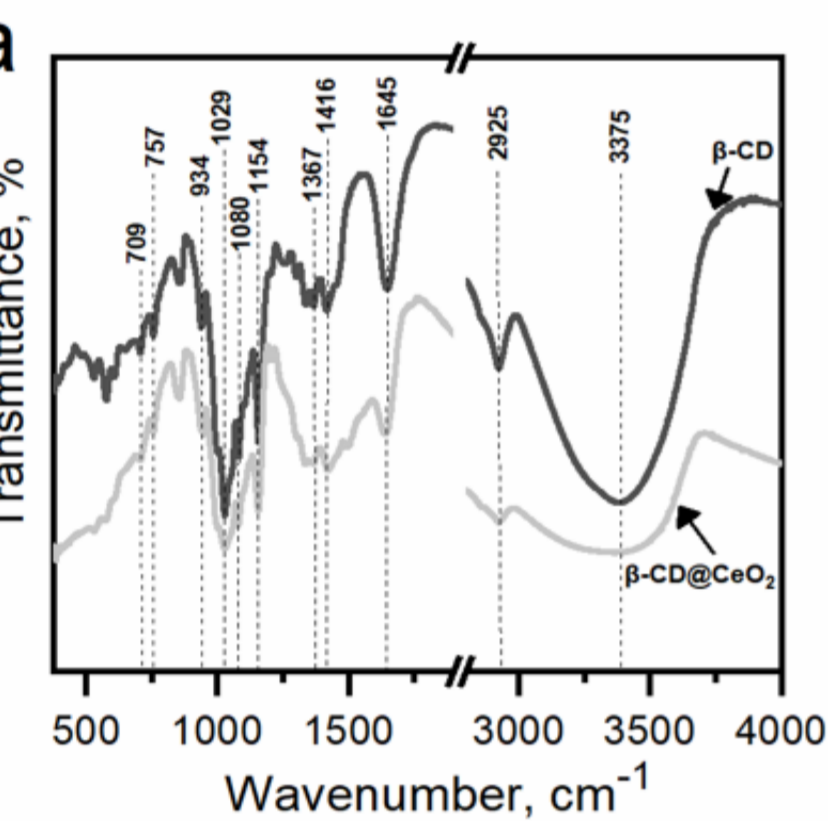


Fig. 2a shows FTIR spectra of β -cyclodextrin and β -CD@ CeO_2 NPs. The intense bands at 3375 cm^{-1} can be assigned as the ν (O-H) mode of H-bonded water molecules and peak at 1645 cm^{-1} can be assigned as δ (O-H). Bands appeared at 1367 cm^{-1} (bending mode of CH₂), 1154 cm^{-1} (asymmetric C-O-C stretching), and 1029 cm^{-1} (C-O stretching) in free β -CD and in the β -CD@ CeO_2 NPs. Compared with free β -CD, the decreased relative intensity of the bands at 934 cm^{-1} (skeletal vibration of 1.4 link bond), 757 cm^{-1} (ring vibration), and 709 cm^{-1} (pyranose ring vibration) in the β -CD@ CeO_2 NPs were attributed to the adsorbed of the β -CDs on the nanoceria surface that prevented the pyranose ring and skeletal vibration. Also, all the significant peaks of β -CD in the range of $700\text{--}1000\text{ cm}^{-1}$ are present in the spectrum of β -CD@ CeO_2 NPs with a small shift. Thus, all the above results indicate that β -CD molecules are adsorbed on the nanoceria surface.

Fig.2: a) FTIR spectra of β -CD@ CeO_2 NPs and β -CD

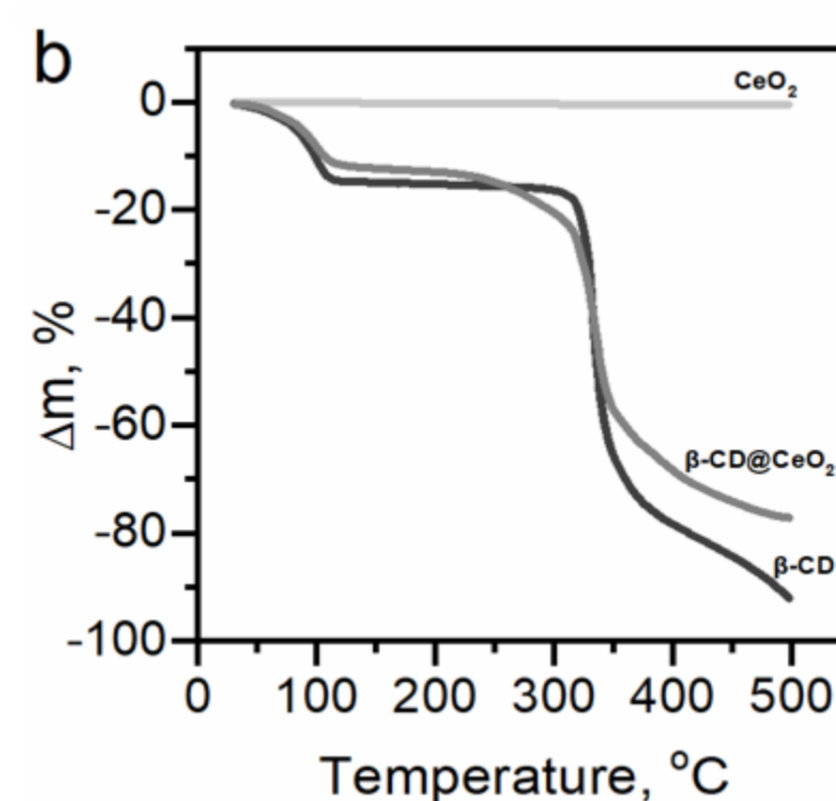


Fig.2: b) TGA curve of β -CD@ CeO_2 NPs, CeO_2 and β -CD

Fig. 2b shows the TGA curves of β -CD at constant heating levels, and its weight loss has been observed in two phases. The initial weight loss at $30\text{--}120\text{ }^\circ\text{C}$ was due to the release of water molecules intercalated with β -CD molecules. The second weight loss was observed in the range of $295\text{--}500\text{ }^\circ\text{C}$ due to the rapid decomposition of monosaccharide units. The residual percentage of β -CD molecules at $500\text{ }^\circ\text{C}$ is 88.0%. The β -CD@ CeO_2 NPs underwent weight loss in two stages as well: the first stage ($30\text{--}120\text{ }^\circ\text{C}$) was due to the loss of water physically linked to the OH groups through hydrogen bonds in the cavities of β -CD, while the second one ($260\text{--}500\text{ }^\circ\text{C}$) was attributed to the decomposition of the β -CD macrocycles. At the same time, unmodified CeO_2 did not lose mass in this temperature range. The percentage of degradation in the first and second step was 15.0% and 62.0%, respectively. This weight loss in β -CD@ CeO_2 is steeper than for β -CD alone that can be considered as an evidence of a loss of stabilized agent (β -CD) adsorbed on the surface of CeO_2 .

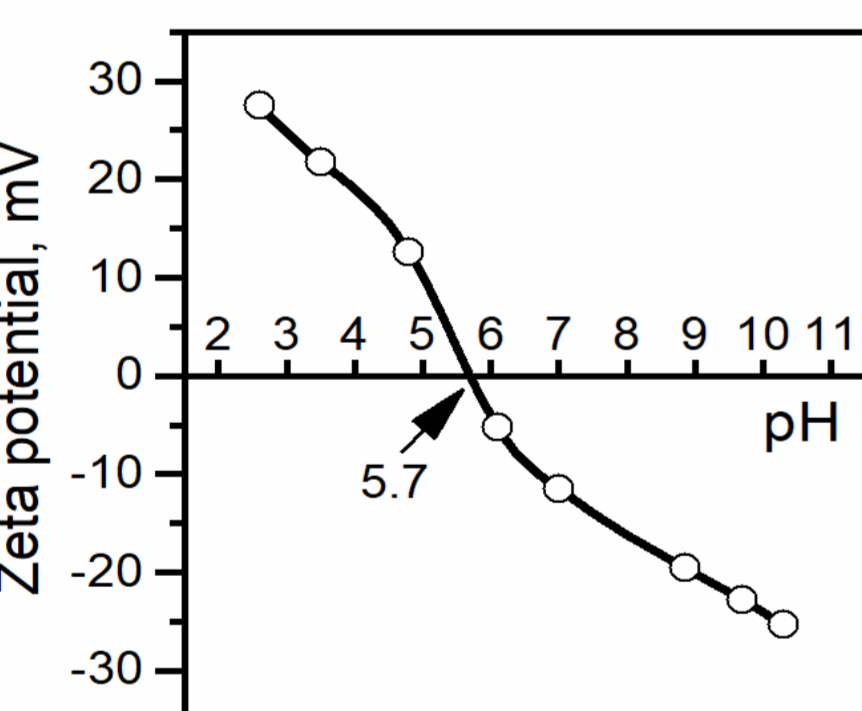


Fig. 3. pH dependence of zeta-potential for β -CD@ CeO_2

As shown in Fig. 3 the zeta potentials of β -CD@ CeO_2 continuously increased with the decrease in pH because of the protonation of the hydroxyl groups of β -CD@ CeO_2 . The IEP of the naked CeO_2 particles according to the literature values is 7.6. After β -CD adsorption on the nanoceria surface, the IEP drastically shifts from 7.6 to 5.7 due to the hydroxyl groups present on the outer surface of the β -CD, which influences the surface charge with the change in pH of the solution. This fact also confirms the adsorption of β -CD and reveals that the β -CD@ CeO_2 is positively charged at $\text{pH} < 5.7$.

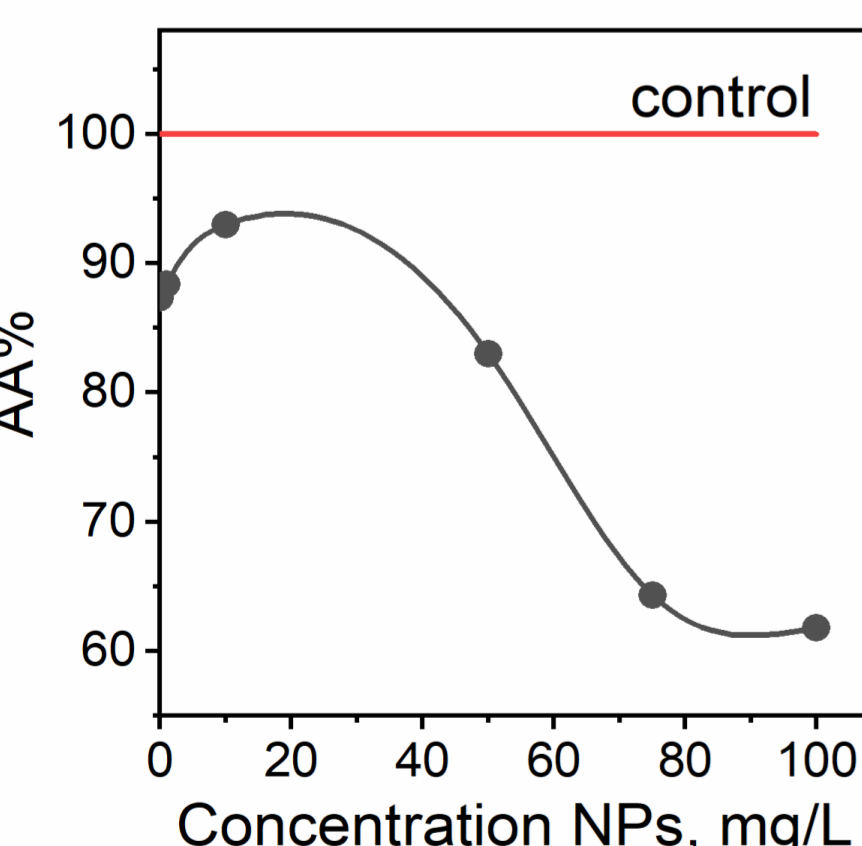


Fig. 4. Inhibition of $O_2^{\cdot-}$ in the presence of β -CD@ CeO_2 NPs at the epinephrine autoxidation

To determine the ability of nanoparticles to carry out a role of SOD mimetic, epinephrine-adrenochrome autoxidation reaction (which is accompanied by the formation of superoxide radicals) was used. In the presence of SOD, which inactivates superoxide radicals, the adrenochrome content decreases. The nanocrystalline cerium oxide, containing on the surface as Ce^{4+} , so Ce^{3+} ions possesses a similar effect. When β -CDs@ CeO_2 NPs are added, inhibition of epinephrine autoxidation is observed, and an increase in the concentration of nanoparticles leads to an increase in the inhibition effect. We have found that β -CDs@ CeO_2 have maximum activity at a concentration of NPs of 100 mg/l.

Table. Size distribution of β -CD@ CeO_2 NPs in the biological media

Colloidal solution (0.25 g L^{-1} β -CD@ CeO_2)	Mean hydrodynamic diameter (nm)
β -CD@ CeO_2 NPs in deionized water	30.2
β -CD@ CeO_2 NPs in 5% glucose	39.7
β -CD@ CeO_2 NPs in 50 mM Tris	38.5
β -CD@ CeO_2 NPs in 20 mM Hepes	47.1
β -CD@ CeO_2 NPs in 0.9% NaCl	1823.4
β -CD@ CeO_2 NPs in FBS (10%)	85.0
β -CD@ CeO_2 NPs and FBS (10%) in 0.9% NaCl	81.1
β -CD@ CeO_2 NPs in PBS	203.3
β -CD@ CeO_2 NPs and FBS (10%) in PBS	36.6
β -CD@ CeO_2 NPs in DMEM	2685.5
β -CD@ CeO_2 NPs and FBS (10%) in DMEM	33.2
β -CD@ CeO_2 NPs in C199	3036.5
β -CD@ CeO_2 NPs and FBS (10%) in C199	83.1

As shown in Table adding of FBS to the solution leads to a certain increase of hydrodynamic diameter of NPs, and in the presence of 0.9% NaCl, DMEM, C199 and PBS diameter remains almost unchanged, indicating the formation of stabilizing layer on the particle surface. The stabilizing layer was formed on the particle surface by adsorption of serum components. Individual serum proteins (with exception of albumin) can play the role of stabilizers.

Conclusions

In this work, β -CD@ CeO_2 NPs are successfully synthesized through a facile method. The characterization by transmission electron microscopy revealed β -CD@ CeO_2 NPs have an average diameter of 5 nm, while an average hydrodynamic diameter of 30.2 nm in aqueous solution was revealed by dynamic light scattering. The results of Fourier transform infrared spectroscopy and thermogravimetric analysis showed that β -CD was adsorbed on CeO_2 particles. These small β -CD@ CeO_2 NPs have excellent colloidal stability. The high dispersing ability of FBS makes it a generally useful dispersing agent for stabilizing investigated NPs in all different media. We have found that β -CDs@ CeO_2 have maximum activity at a concentration of NPs of 100 mg/l. Comparison of β -CD@ CeO_2 nanoparticles with citrate-stabilized NPs has shown that presence of β -CD has no deteriorating effect on the antioxidant activity of nanoceria making these materials potent both as antioxidant agents and carriers of water-insoluble antioxidant molecules for obtaining of hybrid nanomaterials with improved antioxidant action.