Supplementary Material (ESI) for Chemical Communications

Supporting Information.

Detailed Methods.

Ceria nanoparticles preparation. The ceria nanoparticles were synthesized by wet chemical process. All the chemicals were obtained from Sigma-Aldrich Chemicals Inc. For preparation of sample A, $Ce(NO_3)_3 \cdot 6H_2O$ was dissolved in de-ionized water and proper amount of hydrogen peroxide solution was rapidly added with stirring at about 300 rpm. The solution was then heated at 150^{o}C with continuous stirring to obtain a light yellow colored stable dispersion of cerium oxide nanoparticles. The sample B was prepared by traditional homogeneous precipitation method. $Ce(NO_3)_3 \cdot 6H_2O$ was dissolved in de-ionized water and pre-determined amount of ammonium hydroxide was added to the solution with continuous stirring. The precipitate was separated and dried at 80^{o}C and then calcined at 200^{o}C to obtain larger particle size. The particles were then re-dispersed in acidic water (1.5 pH) to obtain a stable dispersion of nanoparticles.

Transmission Electron microscopy of ceria nanostructures. The particle morphology of ceria nanoparticles was studied using high resolution transmission electron microscopy (HRTEM). Ceria nanoparticles were deposited on a carbon coated copper grid for HRTEM analysis by the dipping method. The HRTEM images of the prepared particles were obtained with a Philips (Tecnai Series) transmission electron microscope operating at 300 keV.

X-ray photoelectron spectroscopy. The surface chemistry of the cerium oxide nanoparticles was studied using X-ray photoelectron spectroscopy (XPS) using a 5400 PHI ESCA (XPS) spectrometer. The base pressure during XPS analysis was 10^{-9} Torr and Mg-K_α X-ray radiation (1253.6eV) at a power of 200 watts was used. The binding energy of the Au ($4f_{7/2}$) at 84.0±0.1 eV was used to calibrate the binding energy scale of the spectrometer. Any charging shift produced in the spectrum by the sample was carefully removed by taking C (1s) position (284.6 eV) as a reference line as shown by Barr and Seal [22]. Deconvolution of the XPS spectra was carried out using PeakFit (Version 4) software.

X-ray diffraction (XRD) analysis. XRD analysis was carried out using X-ray diffraction (Rigaku model) with a Cu K_α radiation and operating conditions of 30 mA and 30 kV. The scanning rate was 0.2 degree/min with a step size of 0.01 degree.

Surface area measurements. The nanopowders of cerium oxide were prepared by drying the the dispersions of samples A and B (preparation procedure given above). BET surface area of the nanopowders was obtained by physical adsorption of N_2 at -196^oC using NOVA 4200e surface area and pore size analyzer by Quantachrome Instruments. Prior to the measurements, the samples were degassed for 3 hrs.

Generation of superoxide. Superoxide ($O_2^{\cdot-}$) was generated by incubation of xanthine oxidase (Sigma, St. Louis, Missouri) with hypoxanthine as substrate as previously described [23]. Hypoxanthine was present in all reactions at a concentration of 5 mM. To determine the level of superoxide generated under these experimental conditions, the reduction of ferricytochrome C (Sigma, St. Louis, Missouri) was monitored (change in absorbance at 550 nm) using a Spectramax 190 UV-visible spectrophotometer. Data was analyzed using Softmax Pro 5 (Molecular Devices, Palo Alto, CA) and SigmaPlot 8.0 (SPSS, Inc., Chicago, IL).

Detection of hydrogen peroxide. Hydrogen peroxide levels were assayed using Amplex Red kit from Molecular Probes (Eugene, OR). Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine) is converted to a fluorescent resorufin compound (excitation peak 571 nm, emission peak 585 nm) in the presence of hydrogen peroxide and horseradish peroxidase. Fluorescence or the resorufin compound was determined in white microplates (96-well) using a Varian Cary Eclipse Spectrofluorometer (Varian, Palo Alto, CA) with excitation at 530 nm and emission at 590 nm. The excitation slit width was 5 nm, emission slit width set at 20 nm and a detector voltage of 400 V.

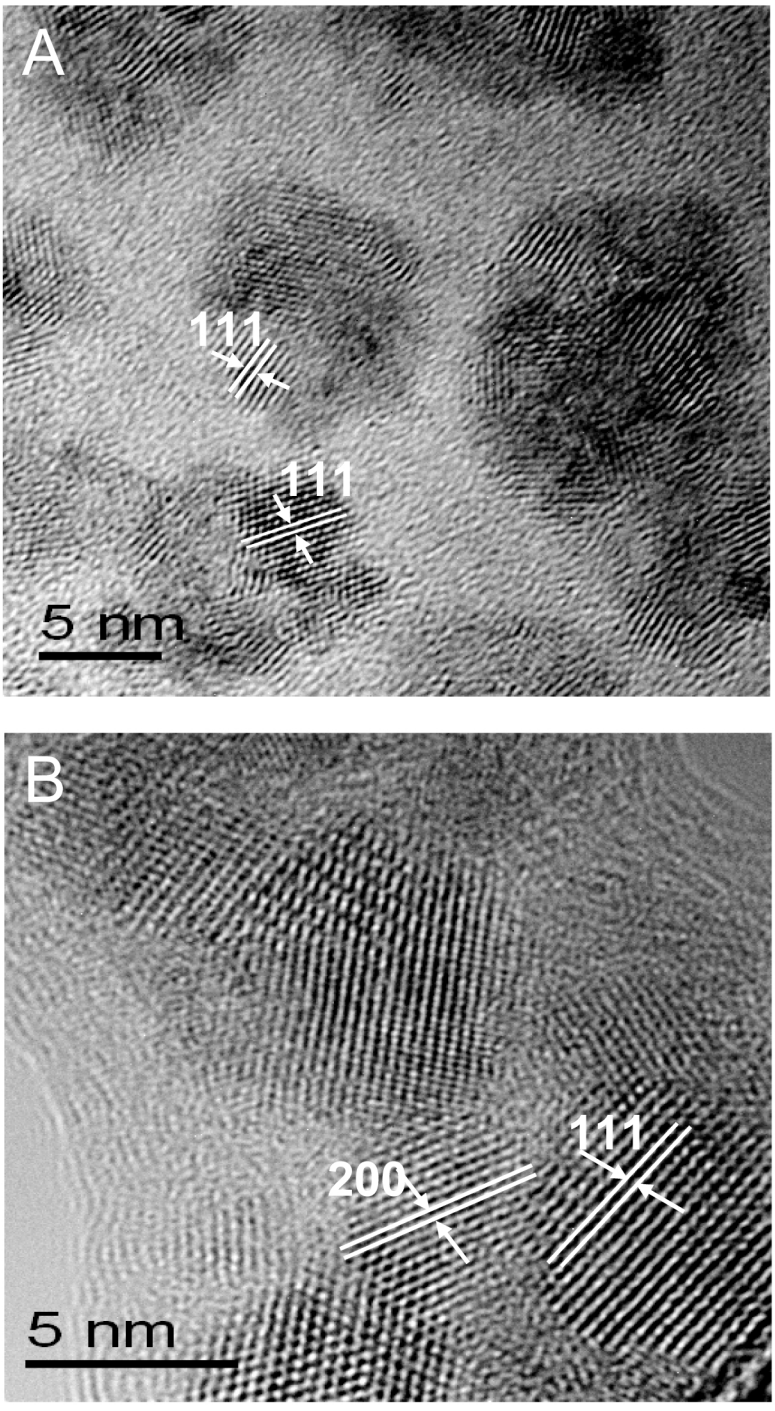
SOD mimetic kinetic assays. Competition for reduction of ferricytochrome C was utilized to assess the kinetics of the SOD mimetic activity of cerium oxide nanoparticles. Superoxide was generated by

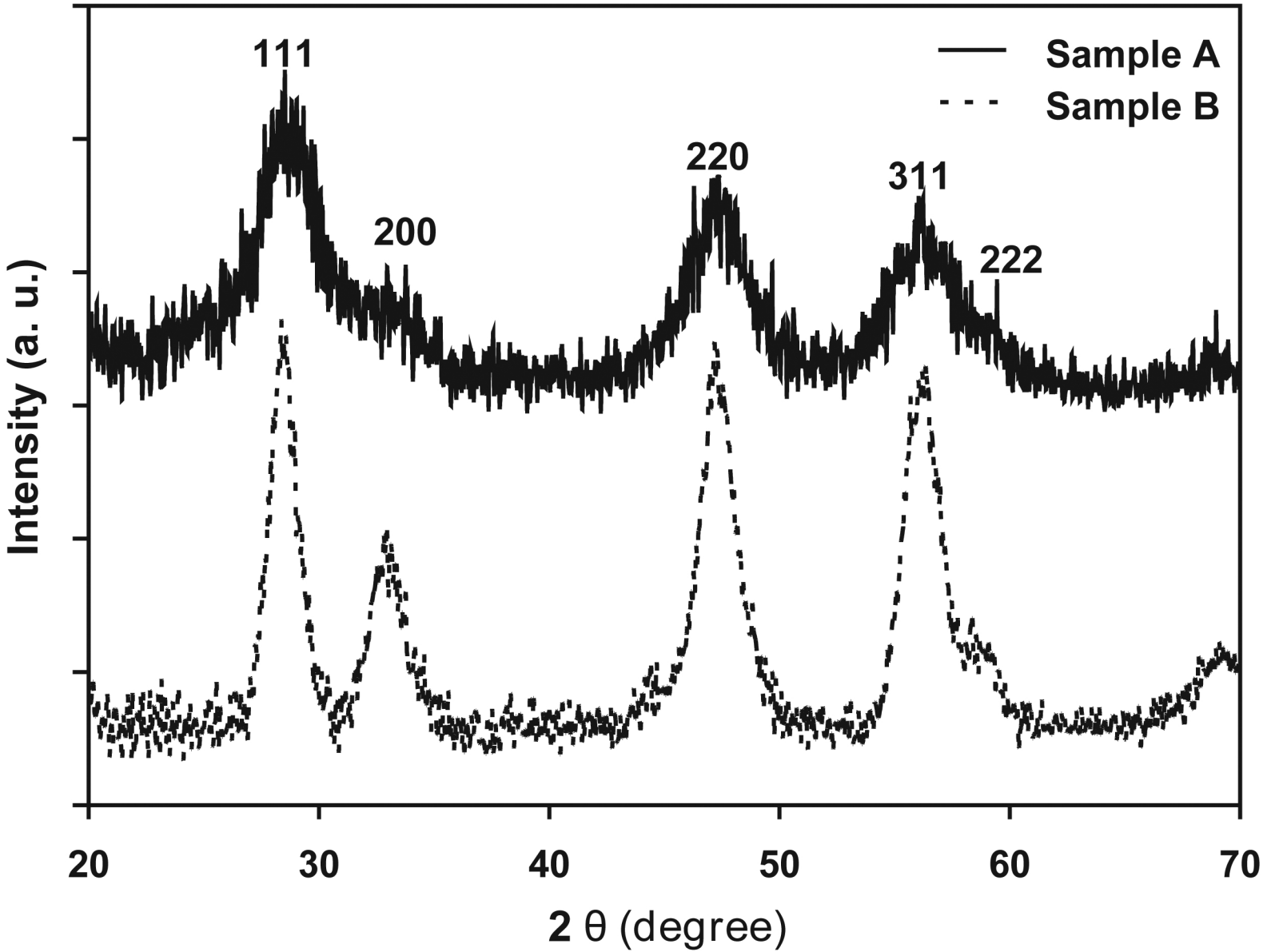
hypoxanthine/xanthine oxidase at a level that resulted in an absorbance change at 550 nm of approximately 0.025 units per minute. Reactions were carried out in 96-well plates and contained 100 µL total volume. CuZnSOD (Sigma, S8160, St. Louis, MO) was utilized as an internal control. Assays were carried out in Tris buffer (50 mM Tris-HCl, pH 7.5) at room temperature and the change in absorbance (550 nm) was monitored using a Spectramax 190 UV-visible spectrophotometer (Molecular Devices, Sunnyvale, CA). In reactions containing lower amounts of superoxide (Δ550 nm < 0.005/minute) no catalase was included. Reactions with higher levels of superoxide (Δ550 nm $\geq$ 0.025/minute) 2,000 units of catalase were added to eliminate the reaction of ferricytochrome C (or nanoparticles) with hydrogen peroxide generated as a result of the SOD mimetic activity.

Supplemental Figure legend.

Figure 1, supplemental. Transmission electron microscopy of nanoceria preparations. HRTEM images of A. CeO_2 nanoparticle sample A indicating particle size of 10 – 15 nm with 3-5 nm crystals present and B. CeO_2 nanoparticle sample B showing particle size of 5-8 nm. The parallel lines observed in each individual ceria nanoparticle are the lattice fringes formed due to periodical arrangement of atoms in the ceria crystal. Arrows indicate the orientation of the nanoparticles on the TEM grid in specific direction found by determining the distance between the two parallel lines (0.312 nm for 111 and 0.270 nm for 200 plane).

Figure 2, supplemental. XRD analysis reveals fluorite crystal structure for both preparations (A and B) of ceria nanoparticles. The XRD spectrum for Sample A shows broader peaks than Sample B indicating smaller particle size in the former. Also, the peaks are relatively sharper in Sample B and the 200 peak is more succinct.


A
111
111
5 nm
B
200
111
5 nm


111
200
220
311
222
Sample A
Sample B
Intensity (a. u.)
20
30
40
50
60
70
2 θ (degree)