

**The excellent peroxidase-like activity of uniform CuCo_2O_4
microspheres with oxygen vacancy for fast sensing of hydrogen
peroxide and ascorbic acid**

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Synthesis of CuCo_2O_4 microspheres

Prior to the synthesis of CuCo_2O_4 microspheres, 1 mmol $\text{Co}(\text{NO}_3)_2$ and 1 mmol $\text{Cu}(\text{NO}_3)_2$ were dissolved in 40 mL ethanol absolute to form a homogeneous solution. Then respectively, 0.5 g PVP and 0.6 g urea were added to the above solution, and some precipitation generated is clearly observed. After that, the solution containing the precipitation was transferred to a Teflon-lined stainless steel autoclave and reacted in oven at 130 °C for 5 h. After naturally cooling the autoclave to room temperature, the products (green precipitation) were separated by centrifugation. The precipitation was washed with ethanol and ultrapure water for several times and dried in a vacuum oven at 60 °C for 12 h. The CuCo_2O_4 precursor (green powders) is successfully collected.

Then the obtained CuCo_2O_4 precursor was evenly spread in porcelain combustion boat. Afterwards the precursor, in the porcelain combustion boat, was calcined at 400 °C for 2 h in air at heating rate of 2 °C•min⁻¹. Finally, the CuCo_2O_4 microspheres were collected for further experiments.

Assay of peroxidase-like activity of CuCo₂O₄ microspheres

The peroxidase mimetic activity of the CuCo₂O₄ microspheres was assessed through an oxidation reaction of a chromogenic substrate (TMB) by H₂O₂. The steps of experiment were described as follows: firstly, adding 200 μL of 0.3 mg/mL CuCo₂O₄ microspheres into a prepared cuvette consisting of 200 μL of TMB (1 mM) and 200 μL of H₂O₂ (0.25 M) together with 1400 μL of phosphate buffer solution (PBS, pH = 5.0). Then, after three minutes of reaction, the reaction was monitored by a UV-1810 PC spectrophotometer. For the purpose of exploring the various response parameters' impacts towards the reaction, the influences of different reaction conditions including pH (2.0-8.0) and temperature (20-60 °C) on the peroxidase-like activity of CuCo₂O₄ microspheres were investigated by the same experiment steps to obtain the optimum reaction conditions. Furthermore, the peroxidase-like activity of the different CuCo₂O₄ microspheres systems was also explored under the same examination conditions.

The kinetic measurements of the catalytic reactions were conducted by recording absorption spectra at 3 min intervals at 652 nm under pH = 5.0 and temperature = 40 °C by changing the concentration of H₂O₂ or TMB. On the basis of the

Lineweaver–Burk double reciprocal plot, according to $\frac{1}{v} = \frac{K_m}{V_{max} \times [S]} + \frac{1}{V_{max}}$, the Michaelis-Menten constant was obtained. In the above equation, v and V_{max} are the initial reaction velocity and the maximum reaction velocity, respectively. K_m is the Michaelis-Menten constant and $[S]$ is the concentration of substrate.

Colorimetric assay of H₂O₂ and ascorbic acid (AA)

H₂O₂ determination: 200 μL of 0.2 mg/mL CuCo_2O_4 microspheres was added into a prepared cuvette consisting of H_2O_2 (200 μL) with different concentrations, 200 μL of TMB (1 mM), and 1400 μL of PBS (pH = 5.0). After reacting for three minutes, the standard curve of H_2O_2 was obtained by recording absorbance of different H_2O_2 concentrations at 652 nm.

AA Determination: A typical reaction system was constructed: 200 μL of CuCo_2O_4 (0.2 mg/mL), 1400 μL of phosphate buffer solution (PBS, pH = 5), 200 μL of TMB (1 mM) and 200 μL of H_2O_2 (0.25M), followed by the addition 200 μL of variable concentrations of AA. The reaction system was monitored at 652 nm by a UV-1810 PC spectrophotometer after 3 min of reaction. The measurements follow the steps of H_2O_2 colorimetric assay, changing only the concentration of additional AA.

Computational theoretical simulative methods:

Materials Visualizer was used for building structure models. The DMol3 package from Materials Studio was used for performing all the calculations. The Perdew–Burke–Ernzerh (PBE) functional of the generalized gradient approximation was selected for performing the unrestricted spin-polarized DFT calculations. The DND basis set (version 3.5) was used, and all the core electrons of Co and Cu were treated using the effective core potentials. The real space cutoff radius was set to 4.1 Å throughout the study. Further, the Brillouin zone was integrated using the Monkhorst–Pack scheme with a grid of $3 \times 3 \times 1$. The convergence criteria for structure optimization and energy calculation were set to (a) an SCF tolerance of 1×10^{-5} hartree, (b) an energy tolerance of 2×10^{-5} hartree, (c) a maximum force

tolerance of 4×10^{-3} hartree/Å; and (d) a maximum displacement tolerance of 5×10^{-3} Å. The bulk crystal of CuCo₂O₄ hybrid was built by replacing two of the six Co atoms of the primitive cell of Co₃O₄ crystal (F 41/D -3 2/M, a=b=c=8.086Å, a=b=g=90°) with Cu atom. After transferring back to conventional cell and cleaving the 220 surface of CuCo₂O₄ hybrid, a vacuum zone with a thickness of 20 Å was added to eliminate any interactions between the adjacent slabs (named as CuCo₂O₄220). The adsorption energies (E_{ads}) of H₂O₂ on the surface are calculated from the relation $E_{ads} = E(\text{molecule} + \text{surface}) - (E_{\text{molecule}} + E_{\text{surface}})$, where E(molecule + surface), E_{molecule}, and E_{surface} represent the energies of H₂O₂ on the CuCo₂O₄220, the isolated H₂O₂ molecule, and the CuCo₂O₄220, respectively. For the adsorption of H₂O₂ on the CuCo₂O₄220 surface with O vacancy, one O atom of CuCo₂O₄220 was deleted to model the O vacancy, resulting to CuCo₂O₄220-O.