Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

2D CoOOH nanosheets as oxidase mimic for colorimetric assay of sulfite in food

Tianxiao Mei, [a,#] Sheng Zhang,[b,#] Jie Sun,[c] and Yihui Hu*[a,b]

[a] Institute for Regenerative Medicine, Shanghai East Hospital, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China

[b] Key Laboratory of Drug Quality Control and Pharmacovigilance, Ministry of Education, China Pharmaceutical University, Nanjing 210009, China

[c] Shanghai Blood Center, Shanghai 200051, China

E-mail: yihuihu2020@163.com

Experimental Section

Chemicals and Instruments. 3,3',5,5'-tetramethylbenzidine (TMB) and sodium hypochlorite (NaClO) were purchased from Aladdin (Shanghai, China). Cobalt chloride hexahydrate ($CoCl_2.6H_2O$), sodium hydroxide (NaOH), sodium sulfite (Na_2SO_3), citric acid monohydrate, and disodium hydrogen phosphate were obtained from Xilong Scientific Co., Ltd. (Guangzhou, China). The required disodium hydrogen phosphate-citric acid buffer solution was prepared according to a standard procedure. All solutions were prepared by using ultrapure water.

Fourier transform infrared spectroscopy (FT-IR) were recorded on a IRTracer-100 Fourier transform infrared spectrometer (Shimadzu, Japan). UV-visible absorption spectra were measured on an Epoch2 microplate reader (BioTek Instrument, Inc., America). X-ray diffraction (XRD) were collected at room temperature on a X'TRA diffractometer (ThermoFisher Scientific Co., Ltd., America). The hydrodynamic diameter and zeta potential were carried out on a Zetasizer Nano ZS90 (Malvern Panalytical, England). Morphological characterization was performed on a Zeiss Ultra 55 scanning electron microscope (Zeiss, Germany) and a JEOL JEM-2100 transmission electron microscope (JEOL, Japan). X-ray photoelectron spectroscopy (XPS) was collected on a PHI 5000 VersaProbe XPS microscope (UlVAC-PHI, Japan).

Synthesis of CoOOH nanozyme. The CoOOH nanosheets (CoOOH NSs) were prepared according to the reported method with slight modification.[1] In brief, 1 mL of sodium hydroxide (NaOH, 1.0 M) was added into 4 mL of $CoCl_2$ (10 mM) solution, the mixture was sonicated for 1 min. Then, 200 μL of sodium hypochlorite (NaClO, 0.9 M) was added to the sonicated mixture, and the mixture was sonicated for another 10 min. The crude products were obtained by centrifugation at 6000 rpm for 5 min, and purified by dialyzing with Mw3500's dialysis bag for 24 hours. The purified CoOOH NSs were obtained by re-centrifugation, and dispersed in 2 mL of ultrapure water with the concentration ~1 mg mL^{-1} for further using. The whole synthesis process was carried out at room temperature (20°C). As well as, the synthesized CoOOH NSs can be dried in vacuum oven for characterization.

Oxidase-mimicking activity measurements. For the oxidase-mimicking activity measurement, we controlled the conditions of temperature, pH, and concentration of CoOOH NSs. In brief, different concentrations (0, 5, 10, 20, 50 and 100 μg/mL) of CoOOH NSs incubated with different temperature (10 - 60℃) was dissolved in 1 mL of disodium hydrogen phosphate-citric acid buffer solution with different pH (4.0 - 10.0) in the absence or presence of 10 μM freshly prepared sodium sulfite, and each of the three groups was only changed one condition. After sufficient mixing for 30 min, 500 μM freshly prepared TMB solution was added to the mixtures. After incubated for another 5 min, the absorption curve or absorbance at 652 nm of obtained solution was measured by UV-vis spectrophotometer to compare which condition could make the difference of absorbance in the appropriate range.

Static kinetic of oxidase-mimicking CoOOH nanozyme. The kinetic experiment of oxidase-like CoOOH nanozyme was carried out at room temperature (20℃) with 20 μg/mL CoOOH NSs dissolved in 1 mL of disodium hydrogen phosphate-citric acid (126.3 mM-36.85 mM, pH 6.0) buffer solution in the presence of freshly prepared TMB with various concentrations. Subsequently, the kinetic measurement was carried out in time course mode by monitoring the absorbance

change at 652 nm for 3 minutes using an UV-vis spectrophotometer. The Michaelis-Menten constant was calculated by using a Lineweaver-Burk plot: $1/v = K_m/V_{max}(1/[S]) + 1/V_m$, where v is the initial velocity, V_{max} is the maximal reaction velocity, and [S] is the substrate concentration. K_m is the Michaelis-Menten constant, which is an indicator of enzyme affinity for its substrate.

Detection of sulfite based on the CoOOH nanozyme colorimetric sensor.

For the sensitive colorimetric detection of sulfite, the sensing procedure is described as follows, unless otherwise stated. First, the oxidase-like activity of CoOOH NSs was investigated at room temperature (20℃), and 20 μg/mL of CoOOH NSs was dissolved in 1 mL of disodium hydrogen phosphate-citric acid (126.3 mM-36.85 mM, pH 6.0) buffer solution in the presence of 500 μM freshly prepared TMB. A spectrophotometer was used to monitor the formation of blue oxidation products (TMB_{OX}). Then, 100 μL of CoOOH NSs solution (200 μg/mL) and 50 μL of sodium sulfite solution with various concentrations were added to 800 μL of disodium hydrogen phosphate-citric acid (126.3 mM-36.85 mM, pH 6.0) buffer solution. After sufficient mixing, the obtained solutions were incubated at 20 ℃ for 30 min. Subsequently, 50 μL of freshly prepared TMB solution (10 mM) was added to the mixtures. The reaction system was incubated for another 5 min at 20 ℃. The absorbance of obtained solution at 652 nm was measured by UV-vis spectrophotometer to quantify the level of sulfite. Each test was repeated 3 times, and the linear relationship to the sulfite concentration was established through the absorbance measurement results.

Detection of sulfite in food. Sugar, biscuits and beer were all purchased from the market in Nanjing, China, and used to prepare for the detection of sulfite by the following treatment. 10 ml of beer was transferred to a centrifuge tube and adequately shaken. After removing the bubbles above the liquid level, 2 ml of obtained liquid was diluted 10 times with disodium hydrogen phosphate-citric acid (126.3 mM-36.85 mM, pH 6.0) buffer solution in the presence of 1 mM freshly prepared EDTA. The solid samples (sugar and biscuits) first should to be mashed, and then dissolved into 10 ml of disodium hydrogen phosphate-citric acid (126.3 mM-36.85 mM, pH 6.0) buffer solution after precise weighing 1.0 g. The obtained mixture was filtered with 0.22 μm syringe membrane filter after ultrasonication for 30 min. Subsequently, the filtrate was diluted to 20 ml with disodium hydrogen phosphate-citric acid (126.3 mM-36.85 mM, pH 6.0) buffer solution in the presence of 1 mM freshly prepared EDTA. In this way, we got three kinds of liquid samples to be tested, including beer diluent, sugar solution and biscuit extract. All samples were stored at 4 °C before analysis.[2,3]

The content of sulfite in the actual sample was described below. In brief, 1 ml of sample solution was mixed with 400 μL of CoOOH NSs (100 μg/mL) and 200 μL of disodium hydrogen phosphate-citric acid (126.3 mM-36.85 mM, pH 6.0) buffer solution. After incubating at room temperature for 30 min, 400 μL of freshly prepared TMB solution (2.5 mM) was added to the mixture. The amount of sulfite in actual sample was measured by UV-vis spectrophotometer at 652 nm in incubating another 5 min. The recovery test also required the accurate addition of the same volume sodium sulfite solution (20, 50, 100 μM) to replace the 200 μL of disodium hydrogen phosphate-citric acid buffer solution in the above steps for testing. Every test was replicated for 5 times.

References

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2 H. Tian, J. Qian, Q. Sun, H. Bai, W. Zhang, Analytica Chimica Acta, 2013, 788, 165-170.
3 R.-F. Zeng, J.-S. Lan, T. Wu, L. Liu, Y. Liu, R. J. Y. Ho, Y. Ding, T. Zhang, Food Chemistry, 2020, 318, 126358.

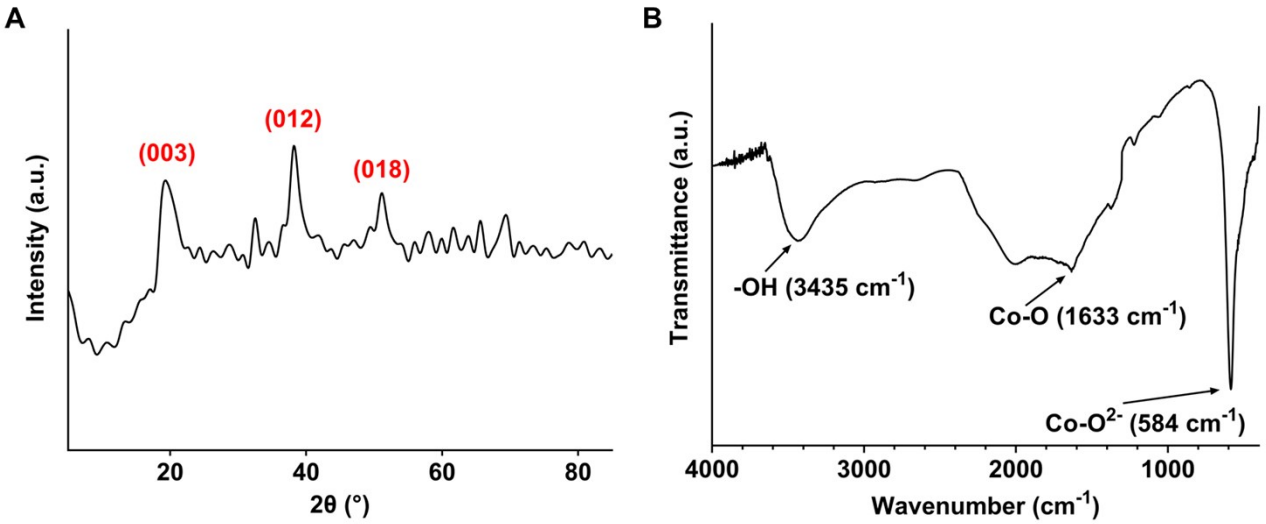

Figure S1. XRD pattern (A) and FT-IR spectra (B) of CoOOH NSs.

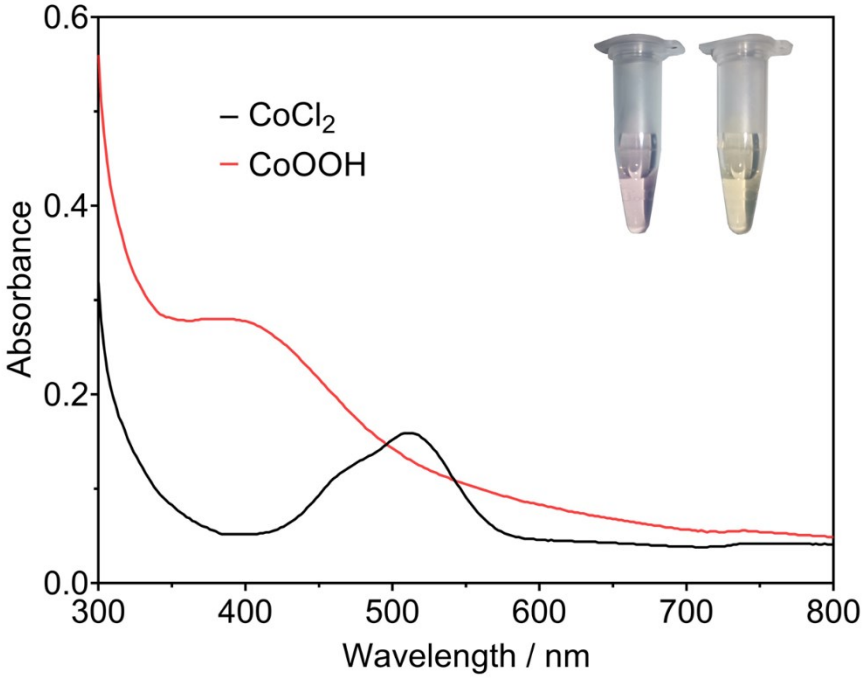

Figure S2. UV-vis spectrum of $CoCl_2$ and CoOOH NSs. Inset represented the corresponding solution photos.

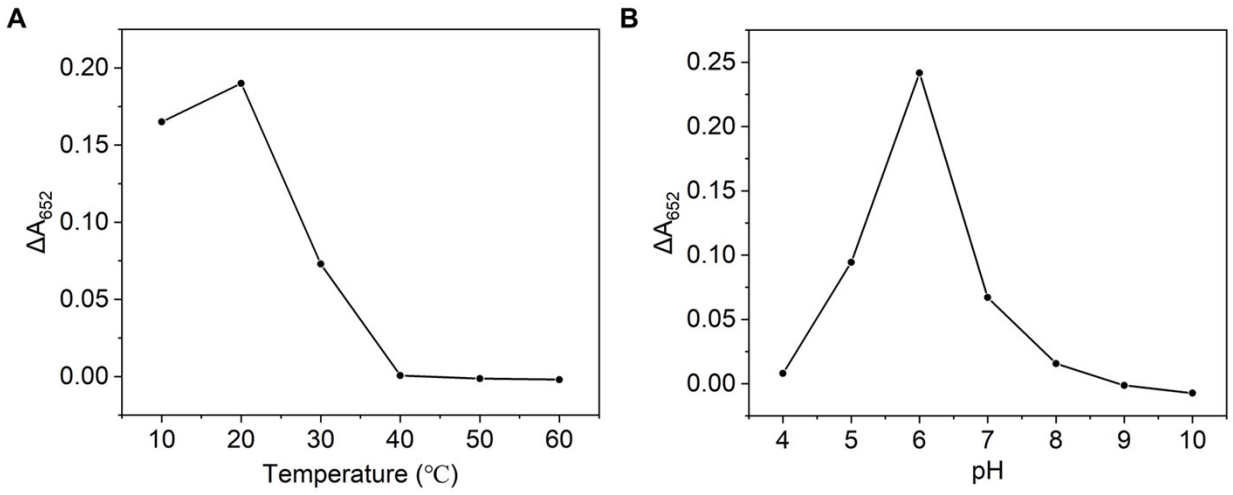

Figure S3. (A) The relationship between ΔA_{652} and different temperature in the range from 10 to 60°C. (B) The relationship between ΔA_{652} and different pH in the range from 4 to 10. ΔA_{652} refers to the absorbance intensity difference of the solutions at 652 nm in the absence or presence of 10 μM of sulfite.

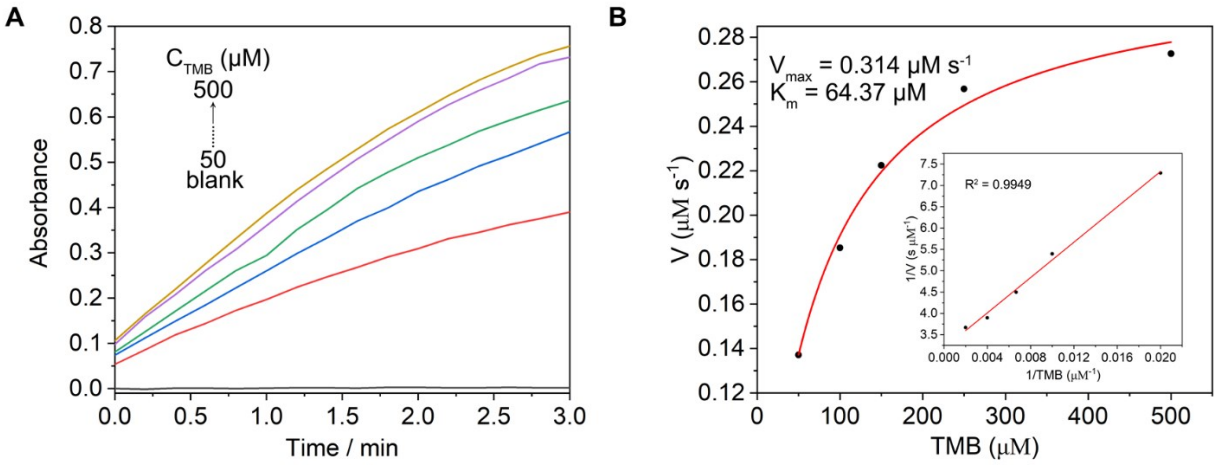

Figure S4. (A) Kinetic curves of A_{652} for monitoring the catalytic oxidation of different concentrations of TMB (from bottom to top: 0, 50, 100, 150, 250 and 500 μM) in the presence of 20 μg/mL CoOOH NSs. (B) Plots of the velocity (υ) of the reaction versus different concentrations of TMB for 20 μg/mL CoOOH NSs catalyzed oxidation of TMB (insert: double reciprocal plots of activity of CoOOH NSs versus varying concentration of TMB).

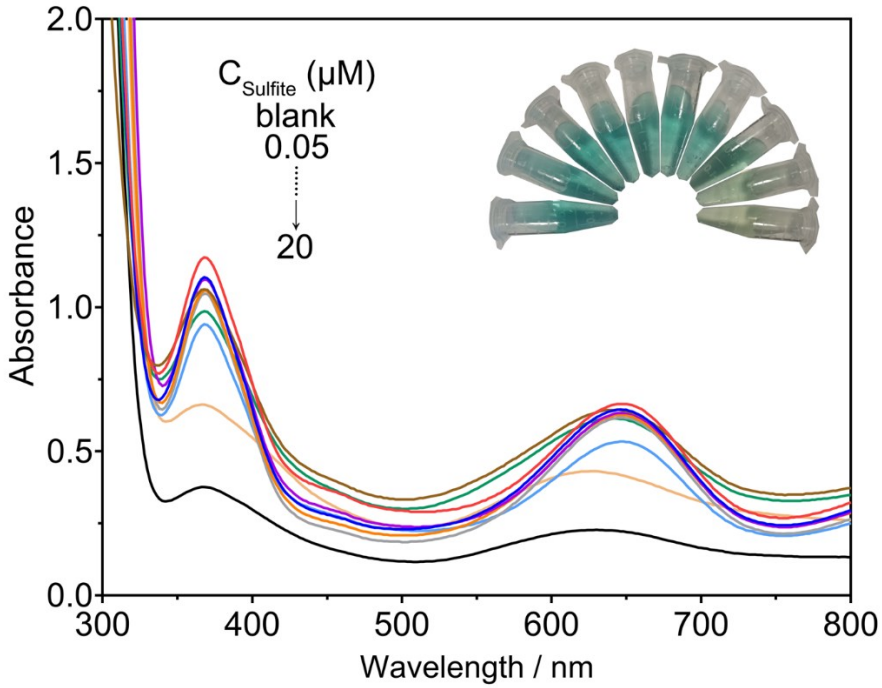

Figure S5. UV-vis spectrum of 20 μg/mL of CoOOH NSs with 500 μM of TMB in the absence or presence of different concentrations of sulfite (from top to bottom: 0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 μM). Inset represented the corresponding solution photo.

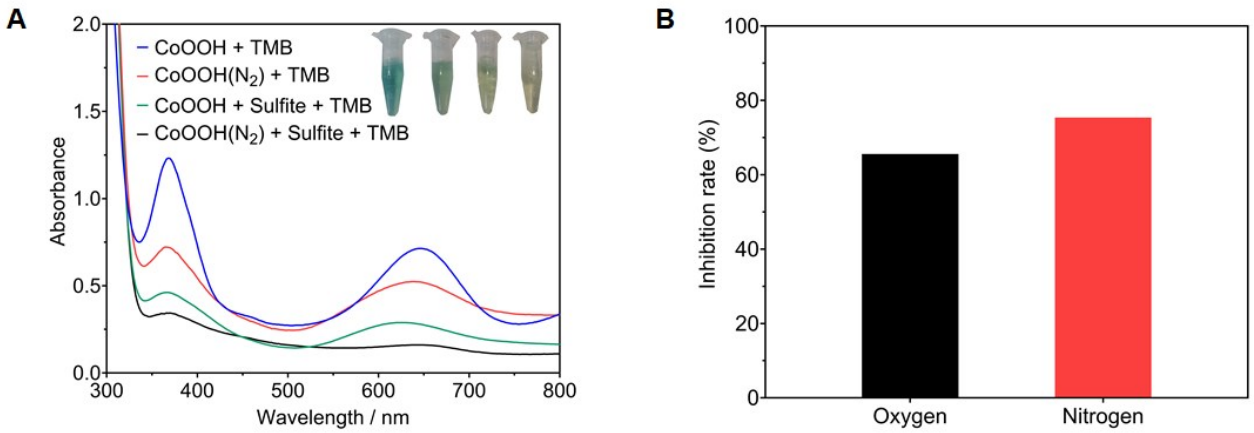

Figure S6. (A) Mechanism of oxidase-like activity of CoOOH NSs explored through UV-vis spectroscopy. Inset represented the corresponding solution photo. (B) The inhibition rate (%) of sulfite to oxidase-like activity of CoOOH NSs within O_2 or N_2. Inhibition rate (%) = $((A_{blank} - A_{sulfite})/A_{blank}) \times 100$.