

Determination of hydrogen peroxide based on calcined layered double hydroxide-modified glassy carbon electrode in flavored beverages

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Abstract In this paper, we developed an amperometric hydrogen peroxide (H_2O_2) sensor based on cobalt-containing calcined layered double hydroxide (Co CLDH). The electrocatalytic activity of the Co CLDH towards the determination of H_2O_2 showed a fast response and high sensitivity. Moreover, the sensor exhibited good reproducibility and long-term stability. The superior electrocatalytic response to H_2O_2 is mainly attributed to the large surface area, minimized diffusion resistance, and enhanced electron transfer of the synthesized Co CLDH. This method with good analytical performance, low cost, and straightforward preparation made this novel electrode material promising for the determination of trace H_2O_2 in beverages with high accuracy, demonstrating its potential for practical application.

Keywords Calcined layered double hydroxides · Cobalt CLDH · Electrocatalysis · Hydrogen peroxide · Food analysis

Jun Wang and Lin Cui equally contributed to this work.

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Introduction

Hydrogen peroxide (H_2O_2) is an indicator of oxidation stress, which is cytotoxic to cellular life. It is not only a by-product of various oxidases in countless biological, but also an essential mediator in food, biology, medicine, industry and environmental analysis [1, 2]. The level of H_2O_2 in living cells is associated with the normal physiologic functions. Therefore, the rapid and accurate determination of H_2O_2 with high selectivity and sensitivity is of great importance in pharmaceutical, clinical, industrial, and environmental analyses [3].

At present, a series of techniques were employed for H_2O_2 analysis, including titrimetry, fluorometry, chemiluminescence, spectrophotometric method, high-performance liquid chromatography, and electrochemical biosensors. Among these procedures, electrochemical technique based on enzyme biosensors has been extensively employed for determination of H_2O_2 with simplicity, high selectivity, and sensitivity [4]. However, the inevitable drawbacks of enzyme biosensors such as the chemical and thermal instabilities originated from the intrinsic nature of enzymes have turned recent efforts to direct determination of H_2O_2 at enzyme-free electrodes. Nowadays, it is becoming more and more significant in analytical chemistry to take advantage of various inorganic nanomaterials because of the following intrinsic advantages: regular structure, chemical and thermal stabilities, high surface reaction activity, high catalytic efficiency, large surface-to-volume ratio, and strong adsorption ability [5]. Therefore, new efforts have been emphasized on these nanomaterials as alternatives for high-efficiency non-enzymatic electrocatalytic applications [6–8]. For example, Dai et al. [9] have used the nanostructure of sheetlike FeS as a peroxidase mimetic to detect hydrogen peroxide. Li et al. [10] constructed non-

enzymatic electrochemical Cu_xO nanoflowers sensor for glucose detection.

In recent years, layered double hydroxides (LDHs) have received considerable attention because of non-toxicity and high chemical and hydrolytic stability [11], ion exchangers, catalysts or catalyst precursors, sorbents, and antacids [12, 13]. They have been used as amperometric or potentiometric sensors via modification on the surface of electrodes [14–16]. However, the application of LDHs as electroactive materials for amperometric sensors is largely restricted due to the limitation of charge transfer between LDHs and electrode. Therefore, increasing efforts have been devoted to investigating calcination products of LDHs, known as calcined layered double hydroxides (CLDHs), owing to their larger surface areas, high metal dispersion, small crystallite size, stability against sintering, and less diffusion resistance than those of LDHs. After heating at moderate temperatures, a highly active homogeneous mixed oxide with porous structure is obtained from LDHs, which are also known as hydrotalcite-like compounds or anionic clays [17, 18]. Controlling thermal decomposition of LDHs with temperature from 450 to 600 °C will obtain the mixed metal oxides, which have large specific surface areas, high dispersion of M^{2+} and M^{3+} (also known as “non-stoichiometric spinels”) at an atomic level, and synergetic effects between the elements [19, 20]. Moreover, mixed oxides containing highly disperse, small crystals of the reducible metal oxides may show interesting catalytic properties [21]. We have explored the Cu–Mg–Al calcined layered double hydroxide-modified electrode as a mimic peroxidase biosensor, which showed good catalysis to hydrogen peroxide [22]. Following this work, we constructed a new sensor based on the cobalt-containing CLDH to detect hydrogen peroxide. It is known that the cobalt-containing mixed oxide was the effective component in mixed oxide-based catalysts and suitable for redox reactions as cobalt cations can attain variable oxidation states. For example,

cobalt-containing mixed oxides prepared from hydrotalcite-like precursors were tested in the catalytic decomposition of nitrous oxide [23, 24] and in the selective catalytic reduction of NO with ammonia [25].

In this article, a new type of catalyst, the Co CLDH film, was fabricated and electrochemically characterized to explore its catalytic activity for H_2O_2 determination. Tests show that the prepared Co CLDH material possesses a porous structure and large surface areas and exhibits excellent performance for H_2O_2 electro-reduction without loading any enzymes. With high electrocatalytic activity, low detection limit, good stability, and ease of production, the Co CLDH-modified electrode opens up new opportunities for fast, simple, and sensitive analysis of hydrogen peroxide.

Experimental

Reagents and apparatus

H_2O_2 was purchased from Shanghai Biochemical Reagent Co. (China) and used as received. $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, and other chemicals obtained from Chemical Reagent Company of Shanghai (China) were of pure analytical grade and used without further purification. Electrochemical experiments were performed with CHI660C electrochemical workstation (Shanghai Chenhua Co., China) with a conventional three-electrode cell. The working electrode is a bare glassy carbon electrode ($d=3$ mm) or a modified glassy carbon electrode. Saturated calomel electrode and platinum wire were used as the reference electrode and auxiliary electrode, respectively. Scanning electron microscopy (SEM) was performed on a Hitachi S-3000 N instrument (Japan). Powder X-ray diffraction (XRD) patterns were carried out with a Rigaku D/MAX 2200PC X-ray diffractometer (Japan) with Cu K_α radiation ($\lambda=0.154178$ nm, graphite

Fig. 1 a, b The XRD and SEM image of the Co CLDH

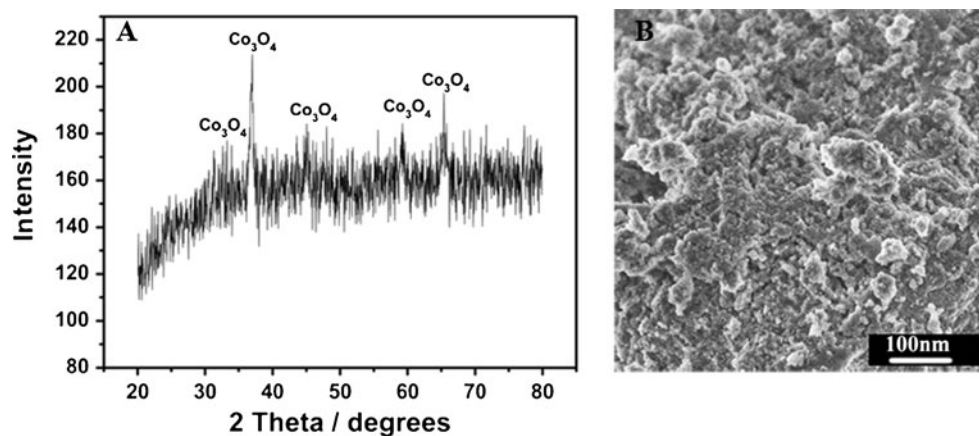
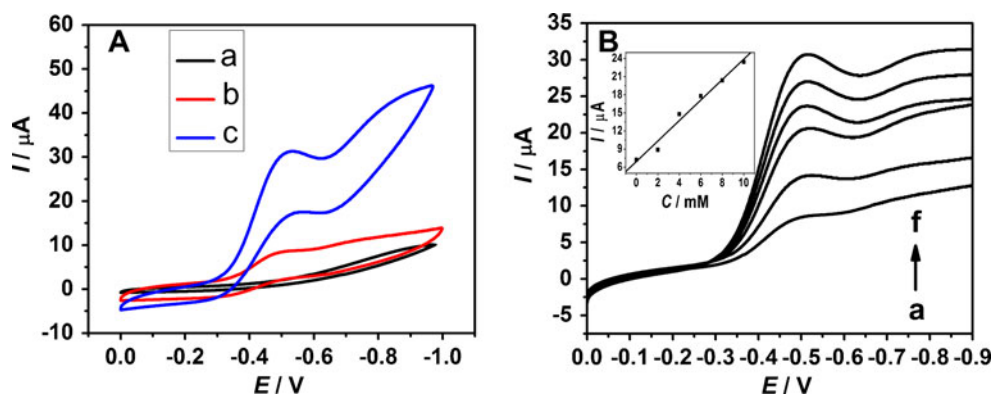


Fig. 2 **a** Cyclic voltammograms of the Co CLDH/GCE in 0.1 M NaOH containing 10 mM H₂O₂ (c), in 0.1 M NaOH (b), and in blank solution (a). **b** CVs of Co CLDH/GCE in 0.1 M NaOH containing (a) 0, (b) 2, (c) 4, (d) 6, (e) 8, and (f) 10 mM H₂O₂ (from a to f) at 100 mV s⁻¹. *Inset*, the linear fitting program of the oxidation peak currents with the H₂O₂ concentrations



monochromator, 28 kV and 20 mA). Additionally, a 2θ range (from 20° to 80°) was investigated at a scanning speed of 10° min⁻¹.

Preparation of Co CLDH

The Co LDH was synthesized by coprecipitation of an aqueous solution of Mg(NO₃)₂·6H₂O, Al(NO₃)₃·9H₂O, and Co(NO₃)₂·6H₂O at room temperature and continuous stirring, with a constant molar ratio Co/Mg/Al of 1:2:1, which has a similar catalytic activity with another ratio [26]. During synthesis, temperature was maintained at 25 °C and the resulting suspension was subsequently kept at 65 °C for 1 h with stirring. The pH was controlled around 10 using NaOH and Na₂CO₃ as the precipitant agent. The resulting suspension was obtained through filtering, water washing, and drying process at 100 °C for 24 h in air. Then, Co-calcined layered double hydroxide was obtained by heating Co LDH in a muffle furnace at 500 °C for 4 h, which was noted as Co CLDH.

Preparation of Co CLDH/GCE

Before modification, the bare glassy carbon electrode (GCE) with a diameter of 3 mm was polished to mirror

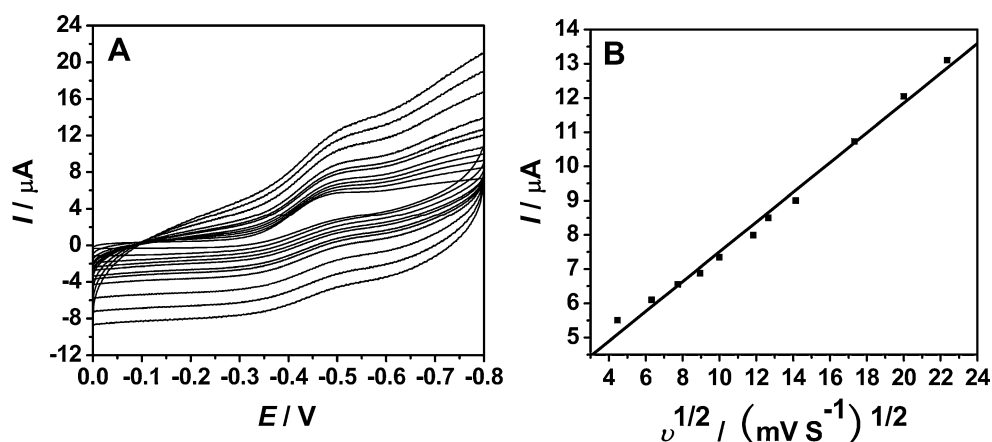
with 0.3 and 0.05 μm alumina slurry on micro-cloth pads in sequence. It was then sonicated in anhydrous ethanol for 3 min and then in double-distilled deionized water for 3 min. Next, it was dried with nitrogen. For preparation of modified electrode, Co CLDH (4 mg mL⁻¹) was first prepared by dispersing Co CLDH in redistilled deionized water, respectively, followed by ultrasonication for 1 h. With a microinjector, 10 μL of 4 mg mL⁻¹ Co CLDH was dropped onto the pretreated surface of glassy carbon electrode, then allowed to dry at room temperature until complete solvent evaporation to obtain Co CLDH/GCE.

Results and discussion

Characterization of the Co CLDH

Figure 1a shows the XRD pattern of the synthesized Co CLDH. It is clear that Co CLDH was composed of Co₃O₄ peaks. Moreover, from the SEM image of the Co CLDH in Fig. 1b, we can see that there are a lot of pores on the surface of Co CLDH, which was obtained by calcining LDHs at 500 °C. According to a previous paper [27], with calcination at 500 °C, LDHs are converted into mixtures of basic oxides. We can see that there are lots of pores on the

Fig. 3 **a** CVs of Co CLDH/GCE in 0.1 M NaOH at various potential scan rates of (from inner to outer) 20, 40, 60, 80, 100, 140, 160, 200, 300, 400, and 500 mV s⁻¹. **b** A linear relationship between the cathodic current and the square root of scan rate



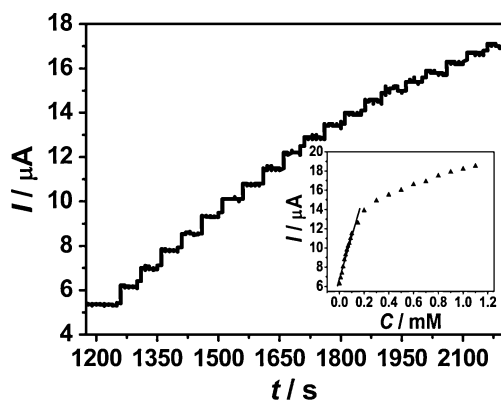


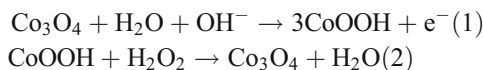
Fig. 4 Amperometric response of Co CLDH/GCE to increasing the H_2O_2 concentration with an applied potential of -0.416 V. *Inset*, corresponding calibration curve

surface of Co CLDH. Upon calcination, sample sintering occurred and broke to smaller pieces and pores appeared due to the loss of water and anions. Co CLDH film could give larger specific surface area, a high surface reaction activity, and an efficient transmission channel for the analyzed molecules to reach the active sites, which will all help to improve the stability and sensitivity of the modified electrode.

Electrocatalytic activity of Co CLDH to H_2O_2

For use of Co CLDH as a biocatalyst for reagentless amperometric non-enzyme H_2O_2 sensor, its electrocatalytic activity toward determination of H_2O_2 was examined. As can be clearly witnessed from Fig. 2a, for the bare GCE, no obvious oxidation and reduction peaks were observed in the potential range of -0.1 – 0 V (curve a). The cyclic voltammograms (CVs) of Co CLDH/GCE in the absence (curve b) and presence (curve c) of 10 mM H_2O_2 were recorded, respectively. When H_2O_2 (10 mM) was added to 0.1 M NaOH, the voltammetric characteristics of the Co CLDH/GCE changed significantly, and the reduction peak current on the Co CLDH electrode increased with a reduction potential at -0.416 V, accompanied by a decrease of the oxidation peak current. The redox couple could be attributed to the

conversion between $\text{Co}_3\text{O}_4/\text{CoOOH}$ species. A possible electrochemical reaction for the H_2O_2 is proposed to be:



The CVs of the Co CLDH-modified GCE in 0.1 M NaOH showed a weak reduction peak at -0.416 V at 100 mV s^{-1} . Upon addition of H_2O_2 , the reduction peak current increased dramatically (Fig. 2b). Clear dependency could be witnessed (inset in Fig. 2b) between the current response and concentration of H_2O_2 in the range of 2 – 10 mM with a correlation coefficient of 0.9902 . The low-cost Co CLDH coating can more effectively promote the transportation and accessibility of target molecules and facilitate the transfer of electrons or signals, which is the key to the much enhanced sensitivity and lowered non-faradic behavior.

Effect of scan rate

To further investigate the characteristics of Co CLDH/GCE, the effect of scan rate on the voltammetric behavior of Co CLDH was studied. Figure 3a shows the CVs of Co CLDH/GCE in 0.1 M NaOH solution at different scan rates (20 – 500 mV s^{-1}). It is interesting to note that the reduction peak potential (E_{pc}) for H_2O_2 shifts negatively with increase in the scan rates, indicating a kinetic control over the reduction of H_2O_2 at the redox sites of the Co CLDH-modified electrode. The cathodic current increases linearly with the square root of the scan rate with a regression equation of $I_{\text{pc}} = 0.4350 v^{1/2} - 3.154$ (μA , V s^{-1} , $R = 0.9955$) in the range of 20 – 500 mV s^{-1} in Fig. 3b, suggesting that it is a diffusion-controlled process within the range of the scan rates investigated [28]. According to the Andrieux and Saveant approach and using Fig. 1 of [29], the average value of k_{cat} is $2.65 (\pm 0.2) \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. These results indicate the high catalytic activity of the sensor for H_2O_2 detection. Figure 1S (see “[Electronic supplementary material](#)”) displayed the relationship between the peak potential (E_{pc}) and the natural logarithm of scan rate

Table 1 Determination of hydrogen peroxide in four flavored beverages

Sample	Value found in diluted samples (mM)	Add H_2O_2 (mM)	Value found after H_2O_2 addition (mM)	R.S.D. (%) ^a	Recovery (%)
orange	0.0065	0.10	0.1061	3.3	93.85
apple	0.01582	0.10	0.1160	3.0	101.1
strawberry	0.01652	0.10	0.1166	2.7	100.5
lemon	0.0085	0.10	0.1081	3.6	95.29

^a R.S.D. (%) calculated from five separate measurements

($\ln\nu$) for Co CLDH/GCE in 0.1 M NaOH containing H_2O_2 . The peak potential (E_{pc}) changed linearly versus $\ln\nu$ with a linear regression equation of $E_{pc} = -0.02097 \ln\nu - 0.3916$; $R = 0.9988$ (ν , mV s^{-1}) in the range from 20 to 500 mV s^{-1} . For a totally irreversible electrode process, the relationship between the potential (E_{pc}) and scan rate (ν) is expressed as follows by Laviron [30]:

$$E_{pc} = E^0 + (RT/\alpha nF) \ln(RT k^0/\alpha nF) + (RT/\alpha nF) \ln \nu \quad (1)$$

where α is the electron transfer coefficient, n is the number of electron, R , T , and F have their usual meanings ($R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$, $T = 298 \text{ K}$, $F = 96,480 \text{ C mol}^{-1}$), and αn was calculated to be 1.224. Generally, α is assumed to be 0.5 in a totally irreversible electrode process. Thus, the number of electrons (n) transferred in the electro-reduction of H_2O_2 is two.

Amperometric response of the proposed H_2O_2 sensor

Figure 4 illustrates the current–time plot for the Co CLDH/GCE with successive additions of H_2O_2 . The inset shows the linear relationship between the catalytic current and the concentration. Under optimized experimental conditions with successive additions of H_2O_2 , the reduction current also increased. As shown in Fig. 4, $5.0 \mu\text{L}$ 10 mM H_2O_2 was injected into 5.0 mL of 0.1 M NaOH at -0.416 V ; the steady-state currents reached another steady-state value (95% of the maximum) in less than 5 s at the Co CLDH/GCE. The electrochemical response to H_2O_2 showed a linear range of 1×10^{-5} – $1.1 \times 10^{-3} \text{ M}$ with the calculated detection limit of $2.0 \times 10^{-6} \text{ M}$ at a signal-to-noise ratio of 3. The sensitivity of the proposed biosensor was estimated to be $44.37 \mu\text{A mM}^{-1}$. The analytical performance of the proposed biosensor was compared with the other sensors reported in the literature. Characteristics such as sensitivity, linear range, and the limit of detection of the biosensors were all summarized in Table 1S (see “Electronic supplementary material”).

Stability and reproducibility of the modified electrode

When the modified electrode was stored in 0.1 M NaOH at $4 \text{ }^\circ\text{C}$ for a week, no obvious decrease in the response to H_2O_2 was observed. After a month, the sensor also retained 95% of its initial response. That was to say, the Co CLDH/GCE had good storage stability. The reproducibility of Co CLDH/GCE electrode was investigated by comparing the amperometric current response to H_2O_2 at six modified electrodes prepared independently, and the relative standard deviation (R.S.D) was 2.3% at a H_2O_2 concentration of 10 mM , which indicated that the modified electrode displayed an acceptable reproducibility.

Real sample measurement

To investigate the application of the sensor, real samples were used to determine the H_2O_2 . The samples were diluted ten times with phosphate buffer solution 7.0. The recovery experiments were also performed. The results obtained of four different flavored beverages were listed in Table 1. We obtained that orange flavor is the one producing the lowest values of the four samples. These results proved that the sensors had potential applications in the determination of H_2O_2 in flavored beverages.

Conclusions

This work has successfully demonstrated a promising H_2O_2 sensor based on the Co CLDH-modified GCE. These experimental results indicate that the Co CLDH/GCE holds the prospect for effective determination of H_2O_2 at low detection limit with very high sensitivity. In summary, ease of fabrication, low cost, and fast response make the H_2O_2 sensor proposed in this study very promising in the pharmaceutical, clinical, and industrial detection of H_2O_2 .

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