



Novel nonenzymatic hydrogen peroxide sensor based on iron oxide–silver hybrid submicrospheres

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ABSTRACT

Fe₃O₄–Ag hybrid submicrosphere was synthesized and developed as hydrogen peroxide sensor in this study. The hybrid sphere was fabricated via a two-step route, and proved by characterizations such as TEM, SEM, EDX, and XPS. Recent studies of hydrogen peroxide sensor based on silver nanoparticles inspired us to study the electrocatalytic property of the as-prepared submicrosphere. Though the Ag amount is quite little in the hybrid spheres, the electrochemical sensor constructed by the hybrid spheres exhibited fast, stable and well-defined electrocatalytic activity towards H₂O₂ reduction, which should be the contribution of the combination of Fe₃O₄ and Ag. The detection limit of H₂O₂ was also found to be 1.2 μM, which was lower than some enzyme-based biosensors.

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1. Introduction

Intensive studies of magnetic nanoparticles have been of much interest due to their prominent properties and potential applications in information storage, drug delivery, and magnetic biosensors [1–5]. Meanwhile, considerable attentions have been paid to noble metal nanostructures for their applications in many areas, such as SERS, catalysis, and biosensing [6–10]. Coating noble metals on magnetic nanoparticles could endow the nanocomposite with multifunctional properties. The typical examples like Fe₃O₄–Ag and Fe₃O₄–Au have become an interesting topic recently [11–16]. Fe₃O₄ was regarded to be biologically and chemically inert in the past. However, Gao et al. recently reported that Fe₃O₄ nanoparticles in fact own an intrinsic enzyme mimetic activity similar to that found in natural peroxidases [17]. Wei et al. made use of this novel property of Fe₃O₄ nanoparticles as peroxidase mimetic to detect H₂O₂ and glucose [18]. The catalytic activity of Fe₃O₄ nanoparticles in these two reports were both investigated by examining color changes of the solution containing various dyes. Zhang et al. presented that Fe₃O₄ nanoparticles can be applied to construct efficient electrochemical sensor to detect H₂O₂ [19]. However, the layer-by-layer assembly is complicated to fabricate.

Herein, we developed Fe₃O₄–Ag hybrid submicrospheres to fabricate an electrochemical sensor to detect H₂O₂. As is known, the rapid and reliable determination of H₂O₂ is of importance due to its application in lots of fields [20–23]. Many enzyme-based biosensors towards H₂O₂ reduction have been made. However, the enzyme-based biosensors were found to have disadvantages, such as the crucial demand on environmental conditions, the high cost and instability of enzymes [24–26]. In recent years, nanomaterials have been introduced to this field. Taking the advantages of catalytic activities, stability and convenience of electron transfer, nanomaterials have been regarded as excellent substitutes for enzymes. Fe₃O₄ nanoparticles are easy to produce and are considered to be more stable over a long time and wide range of temperature compared with enzyme-based sensors [27,28]. Besides that, silver has been studied to exhibit excellent catalytic activity for H₂O₂ in recent years [29,30]. To the best of our knowledge, the catalytic property of magnetite loaded silver nanomaterials has never been reported before. In this paper, we employed Fe₃O₄–Ag hybrid submicrospheres to fabricate the electrochemical sensor towards H₂O₂ reduction to detect H₂O₂. The Fe₃O₄–Ag hybrid submicrospheres were prepared using 3-aminopropyltriethoxysilane (APTES) as a linker, and characterized by TEM, SEM, EDX, and XPS.

2. Experimental

2.1. Materials

Chemicals were all of analytical grade and used as received without any further purification. Water used throughout the

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experiment was ultrapure water with resistivity not less than $18\text{ M}\Omega\text{ cm}$.

2.2. Synthesis of Fe_3O_4 submicrospheres

First, Fe_3O_4 spheres were synthesized according to the literature previously reported with slight modification [31]. Briefly, 0.625 g $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ (2.5 mmol) was dissolved in 20 mL of ethylene glycol to form a clear solution, followed by adding 1.8 g NaAc and 0.5 g polyethylene glycol. The mixture was vigorously stirred for 30 min and then sealed in a 30 mL Teflon-lined autoclave. The autoclave was heated to 200°C and kept for 8 h . The black product was washed and redispersed in 10 mL of water.

2.3. Functionalization of Fe_3O_4 submicrospheres

Functionalization of Fe_3O_4 submicrospheres was achieved by the following steps. Fe_3O_4 aqueous solution (1 mL) was separated by a magnet and redispersed in a mixed solution of trichloromethane (9 mL) and ethanol (1 mL). Then, Fe_3O_4 solution was mixed with APTES ($200\ \mu\text{L}$), followed by sonication for 8 h . The functionalized Fe_3O_4 was separated by a magnet, washed with water for several times and redispersed in 10 mL of water.

2.4. Synthesis of Fe_3O_4 -Ag hybrid submicrospheres

Ag nanoparticles were synthesized as reported [32]. In brief, 20 mL of 1 mM AgNO_3 aqueous solution was mixed with 1.6 mL of 38.8 mM sodium citrate aqueous solution, followed by adding 0.4 mL of 112 mM NaBH_4 aqueous solution dropwise under vigorous stirring. The resulted solution containing Ag nanoparticles was aged for 24 h to decompose the residual NaBH_4 before applied in the subsequent steps.

Fe_3O_4 -Ag submicrospheres were prepared by mixing APTES-functionalized Fe_3O_4 sphere (1 mL) with Ag nanoparticle solution (22 mL). After sonicating the mixture for 30 min , the hybrid spheres were separated by a magnet, washed with water for several times, and redissolved in 1 mL of water.

2.5. Electrochemical measurements

The electrochemical sensor was fabricated by dropping $9.6\ \mu\text{g}$ hybrid submicrosphere onto the polished glassy carbon (GC) electrode, followed by pipetting $10\ \mu\text{L}$ $0.05\text{ wt}\%$ Nafion. Electrochemical measurements were all carried out with a CHI 660A electrochemical analyzer (CH Instruments, Chenhua Co., Shanghai, China), with a conventional three-electrode cell, which includes a Ag/AgCl electrode (saturated KCl) as reference electrode, a platinum wire as auxiliary electrode, and a bare or modified GC electrode (3 mm

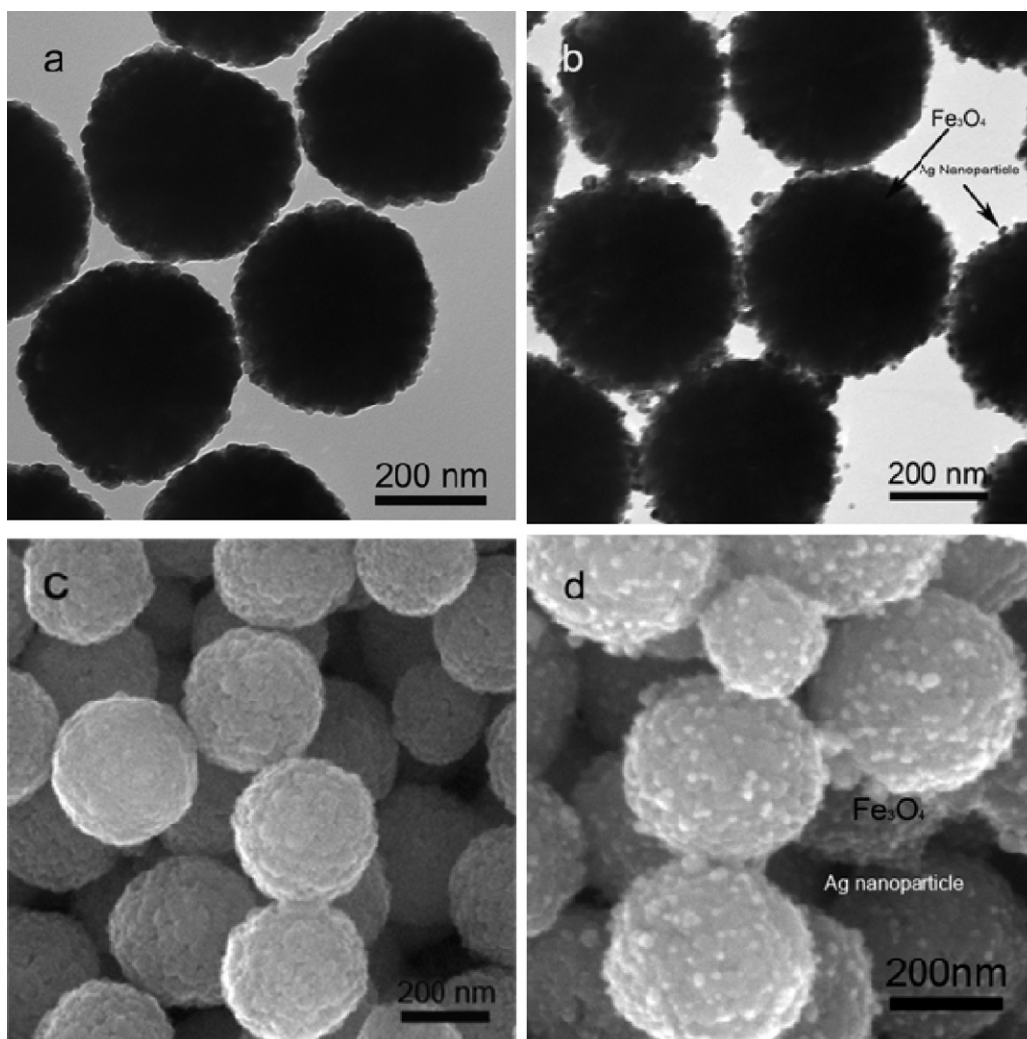


Fig. 1. Typical TEM (a,b) and SEM (c,d) images of Fe_3O_4 submicrospheres (a,c) and Fe_3O_4 -Ag hybrid spheres (b,d).

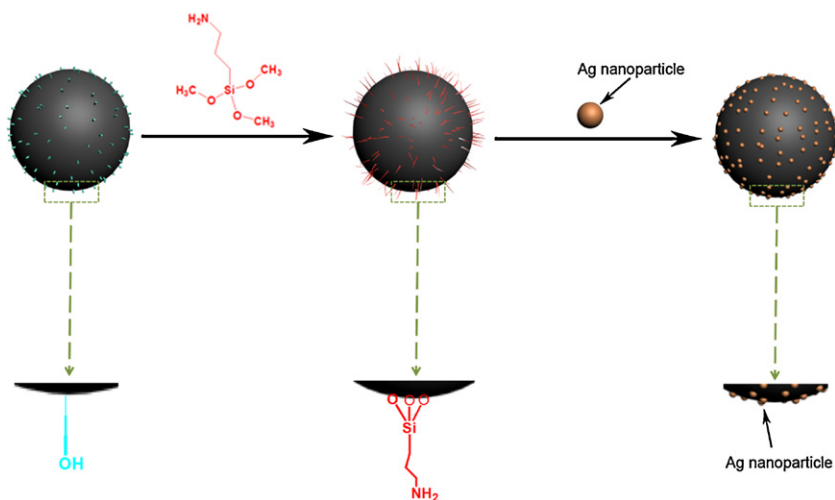


Fig. 2. Schematic illustration of formation process of Fe₃O₄-Ag hybrid submicrospheres.

in diameter, purchased from Inner Mongolia University for the Nationalities) as working electrode. Phosphate buffer solution (PBS, 0.2 M, pH 7.0) is used as supporting electrolyte throughout the electrochemical experiments. Hybrid spheres modified GC electrode was scanned in N₂-saturated PBS containing 4 mM H₂O₂ until the current was stable before all experiments carried out. All solutions were deaerated with high-purity nitrogen prior to and blanketed with nitrogen during all electrochemical experiments.

2.6. Apparatus

TEM images were recorded by a HITACHI H-7650 EM operated at an accelerating voltage of 100 kV. SEM and EDX data were obtained on a XL30 ESEM FEG scanning electron microscope equipped with an energy-dispersive X-ray analyzer at an accelerating voltage of 20 kV. XPS measurements were performed on an ESCALAB-MKII spectrometer (United Kingdom) with Al K α X-ray radiation as the X-ray source for excitation.

3. Results and discussion

Fe₃O₄ submicrospheres were synthesized according to the methods previously reported with slight modifications [31]. Fig. 1a shows the typical TEM image of the prepared Fe₃O₄ submicrospheres, of which the average diameter is estimated to be 400 nm. It can be seen that the surface of the submicrospheres is relatively smooth. The SEM image of Fe₃O₄ submicrospheres was also examined, and the average diameter and smooth surface was also proved by SEM image as shown in Fig. 1c. Since the surfaces of Fe₃O₄ submicrospheres and Ag nanoparticles are both negatively charged, unfunctionalized Fe₃O₄ submicrospheres are not capable of loading Ag nanoparticles, which is in consistency with the literature previously reported [12]. Thus, we applied APTES to functionalize the magnetite submicrospheres. The schematic illustration of formation process of Fe₃O₄-Ag hybrid submicrosphere is presented in Fig. 2. Fe₃O₄ aqueous solution was first separated and redispersed in trichloromethane with the aid of ethanol. As is known, APTES hydrolyzes in aqueous solution and can functionalize the surface of inorganic oxide. However, since APTES applied here is in trichloromethane, the hydroxyl group of which APTES requires to hydrolyze can only be found on the surface of Fe₃O₄ submicrospheres. In this way, APTES hydrolyzes on Fe₃O₄ surface and new Si-O bonds form between APTES and Fe₃O₄ submicrospheres, exposing NH₂ group outside to positively charge the surface of functionalized Fe₃O₄ submicrospheres. Therefore, the

prepared Ag nanoparticles can be easily assembled onto the surface of the functionalized magnetite spheres. Note that the NH₂ group is of importance in the construction, probably due to the strong coordination and/or static interactions between NH₂ groups and negatively charged Ag nanoparticles [12]. Fig. 1b is the TEM image of the as-prepared Fe₃O₄-Ag hybrid submicrospheres. It can be obviously seen that the size of the submicrospheres does not change much, but the surface of the submicrospheres become rougher. Fig. 1d shows the SEM image of Fe₃O₄-Ag hybrid submicrospheres, of which the surface consisted with many nanodots, indicating Ag nanoparticles have been adsorbed onto the functionalized surface of Fe₃O₄ submicrospheres.

The chemical composition of the prepared hybrid submicrospheres was investigated by EDX and XPS. Fig. 3 is the EDX image which shows peaks corresponding to Fe and Ag, and the weight ratio of Ag to Fe₃O₄ is calculated to be less than 1:15. Fig. 4 shows the Fe 2p (a) and Ag 3d (b) regions of XPS spectrum of the hybrid submicrospheres. As shown in Fig. 4a, the peaks at binding energies of 711.1 and 725 eV can be ascribed to Fe 2p_{3/2} and 2p_{1/2} respectively, which are in accordance with those reported previously for Fe₃O₄ [33]. Besides that, the peaks in Fig. 4b centered at binding energies of 368.1 and 374.1 eV can be assigned to Ag 3d_{5/2} and Ag 3d_{3/2} respectively, which are in agreement with metallic silver [34]. Therefore, the prepared submicrosphere can be determined to be Fe₃O₄-Ag hybrid submicrosphere.

Fe₃O₄ and Ag separate nanoparticles are both found to possess the ability to catalyze hydrogen peroxide in previous reports

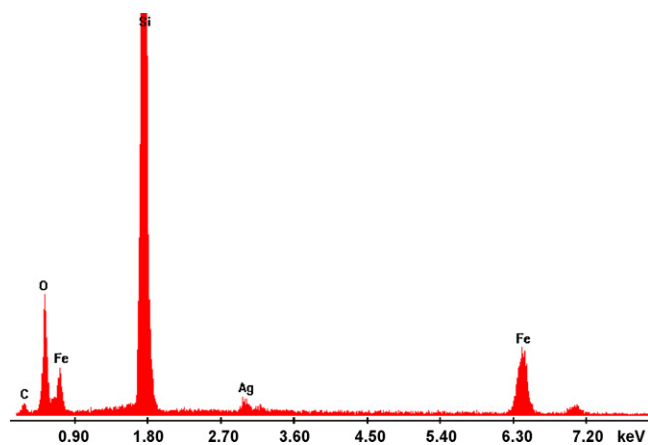


Fig. 3. EDX image of Fe₃O₄-Ag hybrid submicrospheres.

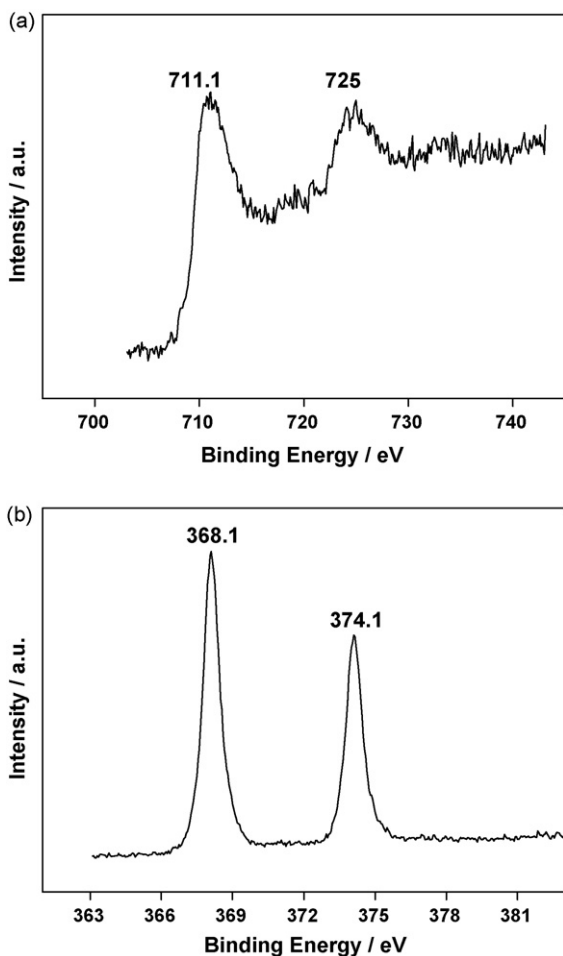


Fig. 4. Fe 2p region (a) and Ag 3d region (b) in XPS spectrum of Fe_3O_4 -Ag hybrid submicrospheres.

[19,29,30]. In this study, Fe_3O_4 -Ag hybrid submicrosphere was modified on GC electrode and applied as electrochemical sensor towards hydrogen peroxide reduction. Curves a and c in Fig. 5 are CVs of Fe_3O_4 and hybrid submicrospheres modified GC electrode in N_2 -saturated PBS without adding H_2O_2 at the scan rate of 50 mV/s. It can be seen that Fe_3O_4 submicrospheres modified

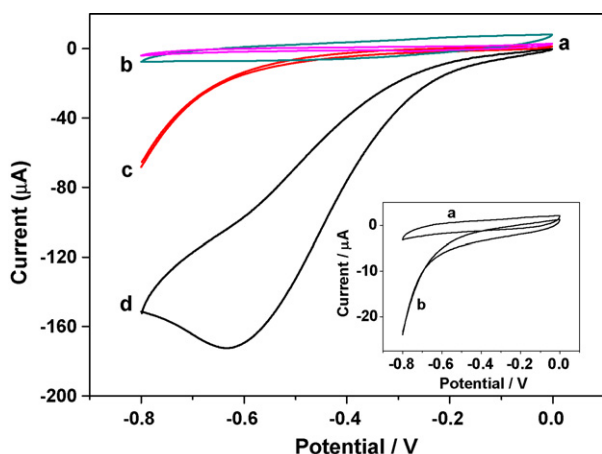


Fig. 5. CVs of Fe_3O_4 (a,b) or hybrid submicrospheres (c,d) modified GC electrode in N_2 -saturated PBS in the absence (a,c) and presence (b,d) of 20 mM H_2O_2 at the scan rate of 50 mV/s. Inset: CVs of bare GC electrode in N_2 -saturated PBS in the absence (a) and presence (b) of 20 mM H_2O_2 at the scan rate of 50 mV/s.

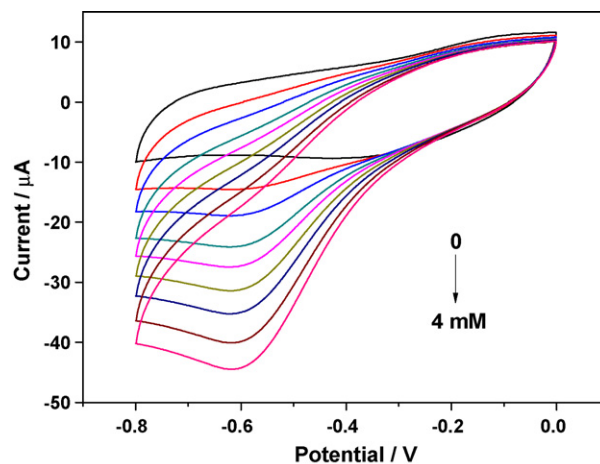


Fig. 6. CVs of hybrid submicrospheres modified GC electrode in N_2 -saturated PBS in the absence and presence of H_2O_2 with different concentrations (from the top: 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 mM) at the scan rate of 50 mV/s.

GC electrode shows current peaks characteristic of Fe_3O_4 [19], and hybrid submicrospheres shows no significant but higher current response compared with bare GC electrode (curve a in Fig. 5 inset) and Fe_3O_4 submicrospheres modified GC electrode. Curves b and d in Fig. 5 show the CVs of Fe_3O_4 and hybrid submicrospheres modified GC electrodes in N_2 -saturated PBS containing 20 mM H_2O_2 at the scan rate of 50 mV/s. Comparing with bare GC electrode (curve b in Fig. 5 inset), Fe_3O_4 and hybrid submicrospheres modified GC electrodes both show enhanced activity towards reduction of H_2O_2 . Though the Ag amount is quite low in the hybrid spheres, the catalytic response characteristic of Ag [29] can be observed from Fig. 5d. Besides that, the hybrid spheres modified GC electrode exhibits characteristic and much larger catalytic response than pure Fe_3O_4 submicrospheres, showing the little amount of Ag is very important to the increased current response. Furthermore, the catalytic current of the hybrid submicrospheres is comparable with the literature reported previously [30], but the Ag amount here is only 0.6 μg comparing with 25.6 μg in the previous report, even total amount of hybrid submicrospheres is only 9.6 μg , indicating that Fe_3O_4 is also essential to the enlargement of the peak current. We also examined the catalytic activity by changing the concentration of H_2O_2 as shown in Fig. 6. It is found that with the increase of H_2O_2 concentration (from the top: 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 mM), the H_2O_2 catalytic current gradually increases. Furthermore, the catalytic activity of the prepared hybrid submicrospheres is also investigated by varying the scan rates. As shown in Fig. 7, the cathodic peak current increases with increasing the scan rates from 10 to 200 mV/s. The peak current increases in a linear relationship with the square root of scan rates in the range of 10–200 mV/s as shown in the inset of Fig. 7, indicating this process is diffusion-controlled.

Fig. 8 is an amperometric response of the hybrid submicrospheres modified GC electrode on successive injection of 0.1 mM H_2O_2 or 0.5 mM H_2O_2 into the stirring PBS at an applied potential of -0.5 V. The hybrid submicrospheres modified GC electrode responded rapidly when adding H_2O_2 into the stirring PBS. The sensor could achieve the maximum steady-state current within 3 s with a detection limit of 1.2 μM estimated at $S/N=3$, which is lower than certain enzyme-based biosensors [35,36] (the detection limits of Refs. [35,36] are around 10 μM). The fast response should be attributed to the contribution of the roughened surface of Ag nanoparticles attached on the Fe_3O_4 submicrospheres. Combining with the data obtained from Fig. 8 and calculation, the sensor exhibits linear response to the H_2O_2 concentration in the range of

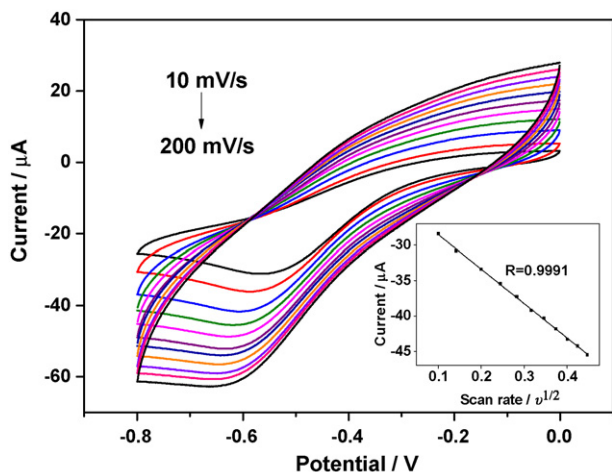


Fig. 7. CVs of hybrid submicrospheres modified GC electrode in N_2 -saturated PBS containing 4 mM H_2O_2 at different scan rates (from the top: 10, 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 mV/s). Inset: plot of electrocatalytic current of H_2O_2 at -500 mV versus $v^{1/2}$.

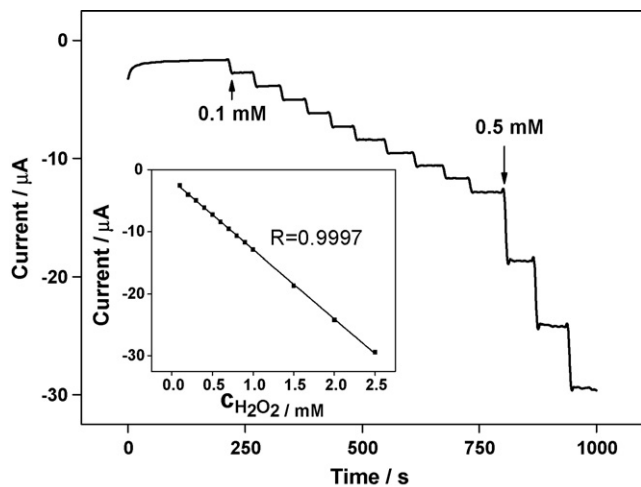


Fig. 8. Typical amperometric response of hybrid submicrospheres modified GC electrode on successive injection of H_2O_2 into the stirring PBS. Applied potential: -500 mV. Inset: plot of H_2O_2 current versus its concentration.

1.2 μ M to 3.5 mM. The reproducibility of the sensor was also investigated. The relative standard deviation (RSD) for H_2O_2 sensing was less than 5% for 6 measurements for the same electrode.

Analysis of real samples has also been investigated. As shown in Table 1, the sensor was applied to the determination of hydrogen peroxide in disinfected fetal bovine serum (FBS). The results show that the recoveries are between 95.4% and 109%. Besides that, the sensor can be well reproduced on different electrodes. Furthermore, the long-term stability of the sensor has also been examined. The sensor was stored in refrigerator at 4 $^{\circ}$ C for 1 month, and no obvious change of current in the response to 1 mM H_2O_2 was found.

Table 1
Determination of hydrogen peroxide in disinfected FBS samples.

Sample ^a	Added (10^{-3} M)	Found (10^{-3} M)	Recovery (%)	RSD (% , $n=6$)
1	1.00	1.03	103	4.50
2	2.00	2.18	109	2.48
3	3.00	2.862	95.4	2.78

^a The samples were diluted 100 times, average of six measurements.

This should be the contribution of the good film forming ability of Nafion [37].

4. Conclusion

In summary, we fabricated a novel electrochemical sensor with Fe_3O_4 -Ag hybrid submicrospheres to detect H_2O_2 . It is found that small amount of Ag incorporated onto Fe_3O_4 could result in largely enhanced response towards H_2O_2 reduction. Besides that, the sensor is fast, stable, with a detection limit of 1.2 μ M. Furthermore, this sensor possesses the function of both Fe_3O_4 and Ag, which may be potential in many fields such as magnetic separation and electrochemical detection.

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