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Research Article

Impedimetric Nanobiosensor Based on Biocatalysis of Catalase at CdTe Nafion Modified Glassy Carbon Electrode for Subnanomolar Detection of Hydrogen Peroxide

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ABSTRACT

A robust and effective nano-composite film based on nafion-CdTe quantum dots modified glassy carbon electrode was prepared by droplet casting. The fabricated nano-composite was used to construct a novel catalase biosensor for the determination of hydrogen peroxide. Direct electron transfer and electrocatalysis of catalase were investigated. A pair of quasi-reversible redox peaks of catalase was observed in 0.20 M deaerated phosphate buffer solution of pH 7.0. The nano-composite film showed a pronounced promotion of the direct electron transfer among catalase and glassy carbon electrode. The immobilized catalase exhibited an excellent electrocatalytic activity towards the reduction of H_2O_2. Cyclic Voltammetry (CV), Chronoamperometry (CA) and Electrochemical Impedance Spectroscopy (EIS) were used to characterize the performance of the prepared nanobiosensor. The results showed that the prepared biosensor could be used as an amperometric biosensor for H_2O_2 detection. The system was also found well suited for the use of impedimetry as an excellent biosensing system for hydrogen peroxide. The electrochemical impedance spectroscopy measurements revealed that the charge transfer resistance decreases significantly after enzymatic reaction with hydrogen peroxide concentration, so that the proposed modified electrode can be applied as an excellent nanobiosensor to the detection of ultra-traces of H_2O_2 (2.0×10^{-10}-2.0×10^{-9} M).

Keywords: Quantum dot; Modified electrode; Impedimetery; Biosensor; Hydrogen peroxide

INTRODUCTION

Semiconductor Quantum Dots (QDs) such as CdTe have been extensively studied in the past two decades due to unique optical and electronic properties which are not available in either discrete or in bulk solids[1,2]. These nanoparticles exhibit quantum size affects including a blue shift of absorption onset, a change in electrochemical potential of band edge, and an enhancement of photocatalytic activities with decreasing crystallite size. Intense interests in QDs focus on their biological applications, which could allow the integration of nanotechnology and biology [3,4].

Recently many uses of Quantum Dots (QDs) in biology were reported, such as cellular labeling [5,6], in vivo tissue imaging, QDs assay labeling [7-9]. QDs-based electrochemical bioassay has also become a favorite topic because of inherent miniaturization, high sensitivity, low cost, and low power requirements. Its ability to promote the direct electron transfer between the biomolecules and electrode surfaces was also explored [10,11]. Recently, QDs have been widely used for the study of the protein electrochemistry and used in biosensor [12-14].

Catalases (EC 1.11.1.6) constitute haem-containing redox enzymes that act on peroxides drastically increasing the rate of disproportion of H_2O_2 into H_2O and O_2. DET among catalase and electrodes have been reported for glassy carbon [15,16], on didodecyldimethylammonium bromide crystal-modified pyrolytic graphite[17]. Polyelectrolyte encapsulated catalase on gold electrodes, polyacrylamide hydrogel-modified pyrolytic graphite [18,19]. On multiwalled carbon nanotubes-nafion in presence of needle shaped DDAB and on nanocrystalline diamond film electrodes [20,21].

Immobilization could modify the protein conformation and thus change enzymatic activity. The most classical way to study the kinetics of H_2O_2 degradation by free or immobilized CAT consists in monitoring the decrease of H_2O_2 concentration during enzymatic reaction by UV spectroscopy. Nevertheless, the specificity, simplicity and inherent miniaturization afforded by the advances in modern electronics have enabled electrochemical sensors and microsystems to rival the most advanced optical methods for

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monitoring bioaffinity reactions [22]. To measure CAT affinity for H_2O_2, some authors have proposed to follow O_2 consumption by the free or immobilized enzyme by amperometry using the specific oxygen electrode [23,24]. Detection by cyclic voltammetry was also reported [25, 26]. Recently, a very performing microfluidic system combined with electrochemical detection has been developed [27].

In this work, a novel impedimetric biosensor for H_2O_2 was prepared by immobilizes CAT and CdTe QDs using Nafion on a GC electrode surface. The results showed that Nafion and CdTe QDs can be successfully used to immobilize CAT, and facilitate direct electron transfer onto the GC electrode surface. Base on the direct electrochemistry of CAT, a H_2O_2 impedimetric biosensor has been fabricated.

MATERIALS AND METHODS

Reagents

Reagents were of analytical grade (from Sigma or Merck) and employed as received. Catalase (EC 1.11.1.6) from bovine liver was purchased from Sigma. All solutions were prepared with double distilled water. The supporting electrolyte used for all experiments was a 0.20 mol L—1 Phosphate Buffer (PB) solution. Hydrogen peroxide standard solution was prepared daily by dilution of the appropriate volume of H_2O_2 (30%, Merck) in PB solution.

Apparatus

The electrochemical experiments were carried out using an Ivium compactstate electrochemical analyzer equipped with a personal computer was used for data storage and processing. All experiments were done in a conventional three-electrode cell using Glassy Carbon (GC) disk electrode as working electrode (with 3 mm in diameter). Auxiliary electrode was a platinum wire and reference electrode was a saturated Ag/AgCl electrode. All measurements were taken at 25°C (± 2°C), temperature maintained with a water thermostatic bath.

Synthesis of CdTe

CdTe quantum dots in the thiol-capped form were synthesized as reported by Zhang et al. with some modifications [28]. Briefly, Tellurium powder was chesen for prepare NaHTe aqueous solution. Te powder was reduced using excessive sodium borohydride in water under stirring and N2 purging. After 8h, solution color was changed from Violet to white and sodium tetra-borate was precipitated. The fresh NaHTe was added to CdCl2 solution containing thioglycolic acid at N2 atmosphere. The molar ratio of Cd2+, Te: TGA was set as 1:0.5:2.4. The pH of solution was adjusted to 11, and then the mixture was heated and refluxed at 100°C for 4 h. The obtained QDs were precipitated with ethanol and then QDs were separated using centrifuge. The resultant precipitate was re-dispersed in water and kept at 4°C in dark.

Preparation of modified electrode

The surface of the GC electrode for each experiment was mechanically polished with 0.05 µm

□-aluminapowder. The polished surface rinsed thoroughly with acetone and then with doubly distilled de-ionized water. Synthesized QDs were added into the 5 mL 0.5% Nafion suspensions. A homogeneous and stable suspension of QDs-Nafion was achieved with the aid of ultra-sonication agitation for about 5 min. Then 50 mg Catalase was added to QDs-Nafion suspension to obtain CAT-QDs-Nafion suspension. The GCE was coated by casting 20 µL suspension of CAT-QDs-Nafion and a beaker was covered over the electrode so that water can evaporate slowly in air and a uniform film can be formed. This enzyme modified electrode was stored at 4˚C in refrigerator when not in use.

RESULTS AND DISCUSSION

Characterization of Synthesized CdTe QDs

From Figure 1A, the UV–Vis absorption spectrum shows that the CdTe QDs solution possesses a well-resolved absorption maximum of the first electronic transition, indicating a sufficiently narrow size distribution of the QDs. It can be seen that the absorption peak occurs at 773 nm (band gap of 2.19 eV) of the CdTe QDs. According to literature [29], the particle size can be determined using the following empirical formula:

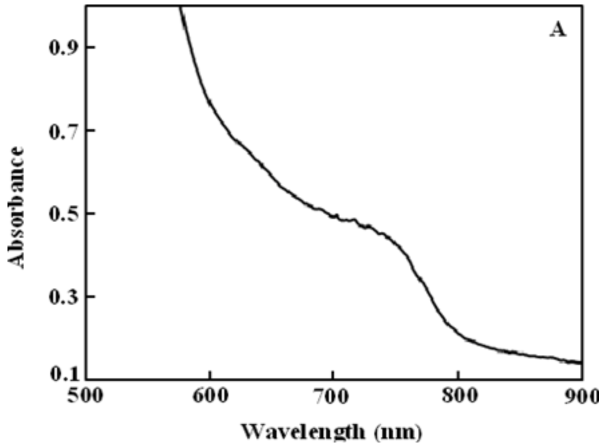

Figure 1: (A) UV–Vis absorption spectrum of CdTe quantum dots.

$$D=(9.8127 \times 10^{-7})\lambda^3 -(1.7147 \times 10^{-3})\lambda^2+1.0064\lambda^{-1}94.84 \quad (1)$$

Where, D (nm) is the diameter of a given QD, and λ (nm) is the wavelength of the first excitonic absorption peak of the UV–vis absorption spectrum. The results show that the mean diameter of the as-prepared CdTe QDs is 11.76 nm.

The CdTe QDs were also studied carefully by TEM imaging (Figure 1B). The morphology and size of the CdTe QDs could be observed clearly. The average size of the studied CdTe QDs was about 10.8 nm, and this value was considered close to the predicted value of 11.76 nm resulting from the empirical formula which seems to be convenient for calculation of the size of CdTe QDs.

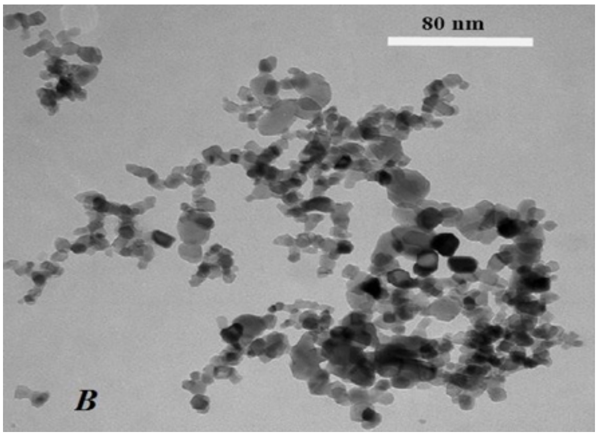

Figure 1: (B) TEM image of Nafion- CdTe-CAT.

Electrochemical behavior of GC/Nafion-CdTe/CAT modified electrode

The electrochemical behaviors of the modified electrode were examined by cyclic voltammetry in a pH 7.0 PBS and the results were shown in Figure 2. On GC/Nafion/CAT no electrochemical response appeared (curve a), which indicated that no electroactive substances existed on the electrode surface. After the CdTe QDs were added into the film, a pair of well-defined quasi-reversible redox peaks appeared (curve b) with the anodic (Epa) and cathodic (Epc) peak potential located at -16 and -93 mV (vs. Ag/AgCl). The formal potential (E0'), which is taken as the average of the anodic and catholic peak potential, was estimated to be –54 mV (vs. Ag/AgCl), and it was corresponded to the active center of CAT Fe(III)/Fe(II) redox couple. This formal potential is 200 mV–400 mV more positive than the catalase formal potential at the surface of other modified electrodes presented in the literature, such as; dimyristoylphosphatidylcholine (DMPC) lipid film −0.426 V[30], poly (amidoamine) dendrimer −0.469 V [31], chitosan −0. 458 V [32], collagen film −0.41 V [33], poly acryl amide hydrogel film −0.459 V [19], didodecyldimethyl ammonium bromide −0.162 V [20], and gold electrode modified with single wall carbon nanotubes −0.414 V [34]. The separation of peak potentials (Δ Ep) was got as 75 mV. The large peak separation of this electrode was in accordance with a similar CAT modified electrode with Nafion and multi walled carbon nanotubes [35], which may be ascribed to the immobilization of protein molecules in an abnormal orientation. The results indicated that the presence of CdTe quantum dot could greatly facilitate the electron transfer of CAT with the substrate electrode and the direct electron transfer of CAT was achieved in the GC/Nafion-CdTe. Figure 3 demonstrated the cyclic voltammograms of the GC/Nafion-CdTe/CAT at various scan rates in pH 7.0 PBS. With the increase of the scan rate the redox peak currents increased gradually and the peak-to-peak separation become greater, which indicated the electrode process turned to more irreversible. The redox peak currents had almost the same values in the selected range of scan rates, which indicated that all the electroactive ferric catalase [CAT Fe(III)]was reduced to ferrous catalase [CAT Fe(II)] on the forward scan and the CAT Fe(II) was re-oxidized to CAT Fe(III) on the reverse scan. The increase of the background current with the increase of the scan rate maybe due to the accessible capacitances of the Nafion at the carbon surface.

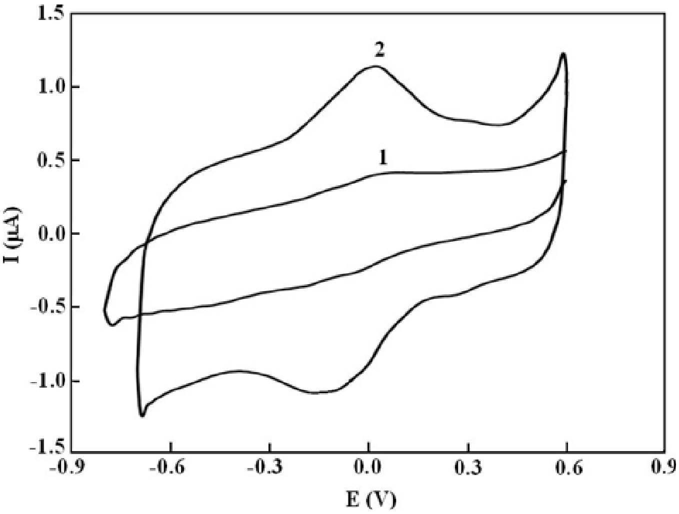

Figure 1: GC/Nafion-CdTe/CAT (b) modified electrode in deaerated phosphate buffer solution (pH 7.0), scan rate 100 mV s-1.

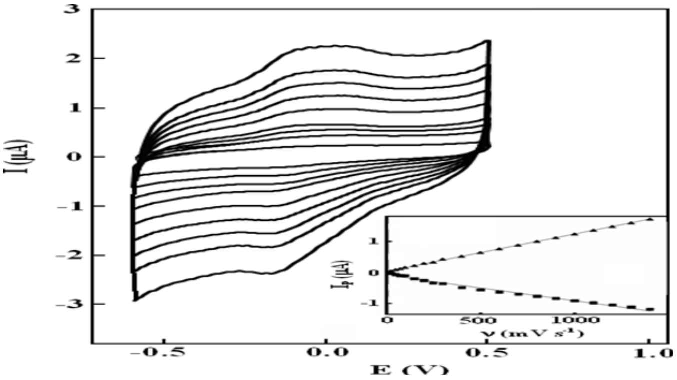

Figure 3: (Cyclic voltammograms of GC/Nafion-CdTe/CAT modified electrode in deaerated phosphate buffer solution (pH 7.0) with varius scan rates in the range of 10 to 300 mVs-1 (from inner to outer). Inset) Relation between scan rate and peak currents of GC/Nafion- CdTe/CAT modified electrode in deaerated phosphate buffer solution (pH 7.0)..

The relationship between the redox peak currents of CAT with the scan rate was further constructed. Both the cathodic and anodic peak currents were proportional to the scan rate, which was the characteristic of diffusion less-confined thin-layer electrochemical behaviors 36. The linear regression equations for anodic and cathodic currents were got as Ip=0.0012ν+0.0369 (n=19, R2=0.9997) and Ipc=-0.0008ν-0.0908 (n=19, R2=0.9856), respectively.

Electrocatalytic reduction of H_2O_2 on the GC/Nafion-CdTe/CAT modified electrode

Due to electrochemical reversibility and high electron transfer rate constant of catalase at the GC/Nafion-CdTe modified electrode, it can be used as a mediator to shuttle electron between electrodes and analyte molecules. Figure 4 shows the cyclic voltammograms of GC/Nafion-CdTe/CAT modified electrode in presence of minimum (10 µM) and maximum concentration (50 µM) of H_2O_2 in pH 7.0 PBS. In the absence of H_2O_2, a quasi-reversible peaks of catalase was observed, which was the same as showed in Figure 2. However, in the presence of H_2O_2, the voltammetric behavior changed. A large cathodic current appeared that shows a typical electrocatalytic reduction process. The ratio of cathodic current in the presence and absence of H_2O_2 can be defined as catalytic efficiency [36]. It was found that the catalytic efficiency decrease with increasing scan rate, and the cathodic current increased with increasing concentration of H_2O_2, which were all characteristics of electrochemical catalysis [37]. As shown in Figure 4, by increasing the concentration of H_2O_2, the catalase cathodic peak current increased while its anodic peak current decreased.

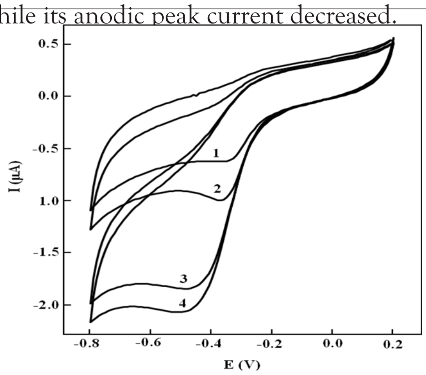

Figure 4: Cyclic voltammograms of the GC/Nafion-CdTe/CAT modified electrode in the phosphate buffer solution of pH 7.0 at 100 mVs-1 in the (a) absence andpresence of 10(b), 40(c) and 50 µM H_2O_2 (d).

Amperometric detection H_2O_2 using GC/Nafion-CdTe/CAT modified electrode

Figure 5A shows the typical amperometric response of the fabricated biosensor to H_2O_2 with the addition of successive aliquots of hydrogen peroxide into continuously stirred PBS at an applied potential of -500 mV. When the modified electrode reached a steady baseline in blank PBS, adding H_2O_2 into the solution caused obvious current response. The aerometric biosensor exhibited rapid and sensitive response to the change of hydrogen peroxide concentration. The cathodic current reached 95% of steady-state current within 15 s. Figure 5B exhibits the typical calibration curve of the biosensors to H_2O_2. The linear range of GC/ CdTe-Nafion/ CAT modified electrode spanned from 0.27 × 10^{-6} to 24.8 × 10^{-6} M (r=0.9965),with a detection limit down to 8.33×10^{-8} M at a signal to nc

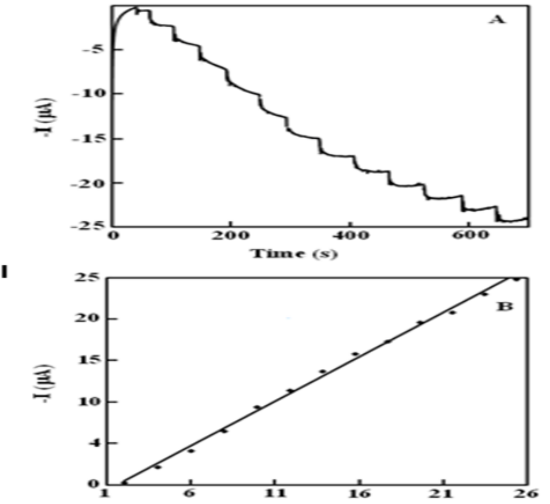

Figure 5: (A) Typical amperometric response of the CdTe-Nafion/ CAT modified electrode to H_2O_2 with the addition of hydrogen peroxide into continuously stirred PBS (applied potential was -500 mV). (B) Calibration curve of the biosensors to H_2O_2.

Impedimetric detection of H_2O_2 using GC/Nafion-CdTe/ CAT modified electrode

Impedance spectroscopy is an effective method to probe the interface properties of surface-modified electrodes [37]. The complex impedance can be presented as the sum of the real, Zre, and imaginary, Zim, components originating mainly from the resistance and capacitance of the cell, respectively. A typical shape of a Faradaic impedance spectrum (presented in the form of a Nyquist plot), includes a semicircle region lying on the Zre-axis followed by a straight line. The semicircle portion, observed at higher frequencies, corresponds to the charge-transfer-limited process, whereas the linear part of the spectrum is characteristic of the lower frequency range and represents the diffusional-limited electron-transfer process. The semicircle diameter in the impedance spectrum equals to the charge-transfer resistance, Rct.

The Electrochemical Impedance Spectroscopy (EIS) in frequently used to determine the amount of redox species and probes that involved with change in the charge transfer resistance and/or capacitance on the electrode surface. The relation between bulk concentration and charge transfer resistance can be described using the following equation [37, 38].

RctT̀RT

Where, kct is potential dependent charge transfer rate constant, [S] corresponds to the concentration of the redox species, and the other symbols have their usual meanings.

The GC/Nafion-CdTe/CAT modified electrode was examined as a biocatalytic interface to product H_2O from H_2O_2 using EIS. It can be considered that both reactions (H_2O_2/H_2O) and (cat-Fe(III)/ cat-Fe(II)), are fast. Also, cat-Fe(III)/cat-Fe(II) reaction is reversible, therefore, the extent of H_2O_2 formation is controlled by the concentration of H_2O_2 and one may replace $[S]=k1[H_2O_2]$, where k1 is a constant. If all other parameters be constant, a linear relation in the form of $1/Rct=k[H_2O_2]$ is simply found, in which k includes all constants. According to this equation, the values of charge transfer resistance decrease with increase in H_2O_2 concentration. The sensivity of 1/Rct as a function of H_2O_2 concentration depends on the magnitude of the applied DC potential [38].

Figure 6 showed the Nyquist plots obtained on GC/Nafion-CdTe/ CAT modified electrode in deaerated phosphate buffer solution (pH 7.0) at -300 mV in different concentrations of H_2O_2. For the clarity, only some Nyquist plots are presented in the Figure 5. The equivalent circuit Rs(RctCPE1) consist of one constant phase element in series with a resistance, was found to give the best fit to the experimental data. The chi-square goodness-of-fit calculated for each fit by the FRA software employed, was systematically checked to validate all of the calculations performed. For all of the cases studied in this work, calculated values of chi-square for the mentioned equivalent circuit were in the range of 0.0001-0.01, much lower than the tabulated value for 5 degree of freedom (67.505 at 95% confidence level), thus demonstrating the high signifi

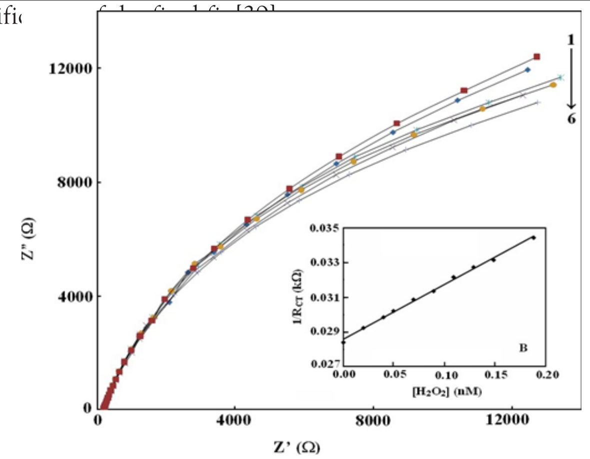

Figure 6: (Nyquist plots obtained on GC/Nafion-CdTe/CAT modified electrode for different concentration of H_2O_2 in a 0.20 M phosphate buffer solution of pH 7.0, EDC=-300 mV, Eac=10 mV and frequency range: 10kHz to 100 MHz. Symbols show the experimental data and lines show the approximated results. Inset) The calibration curve obtained using 1/Rct as a function of H_2O_2 concentration.

The variation of 1/Rct versus H_2O_2 concentration produced a calibration curve (Figure 6B) over a wide linear range of 2.0 × 10^{-10} -2.0 × 10^{-9} M with a regression equation of $1/Rct=0.0314[H_2O_2]+0.0286$ (R2=0.9969) and a limit of detection of 9.63 × 10^{-12} M, as determined from CLOD=3 Sb/m, where Sb is standard deviation of five replicate blank signals and m is slope of the calibration curve. The reproducibility of five different electrodes prepared was examined at a 15 nM concentration of H_2O_2, which resulted in a relative standard deviation of less than 12%. Table

1 compares the linear range and detection limit of the proposed GC/Nafion-CdTe/CAT modified electrode with those of some of the best previously reported voltammetric non-enzymatic and enzymatic hydrogen peroxide biosensors [26,39-47]. As is obvious from the summarized data, the proposed impedimetric GC/Nafion-CdTe/CAT biosensor shows the highest sensitivity and lowest limit of detection in the series [48,49].

Table 1: Comparison of linear range and limit of detection of different electrochemical sensors reported for hydrogen peroxide determination.

Electrode	Linear range	Detection limit	References
GC/CdTe-Nafion/CAT	2.0×10^{-10} -2.0×10^{-9} M	9.63×10^{12} M	-
GC/MWCNT/CAT	10–100 µM	1 µM	(26)
GC/sol–gel–peroxidase	up to 3.4 mM	5×10^{-7} M	(39)
Carbon paste/CAT	0.1 ‑ 1 mM	-	(40)
Au/Cyst/GNP/Hem	3.6×10^{-7} ‑8.6×10^{-4}	1.2×10^{-7} M	(41)
GC/QD/HRP	5×10^{-6}- 1×10^{-4} M	2.8×10^{-7} M	(42)
CP/HRP-GA-BSA	2 nM- 10 µM	1 nM	(43)
GC/MB/SiO2/HRP	1×10^{-5}- 1.2×10^{-3} M	4×10^{-6} M	(44)
GC/MWCNT/Chitosan/HRP	1.67×10^{-5}- 7.4×10^{-4} M	1.3×10^{-5} M	(45)
PG/NHSS/SWNT/HRP	40 nM – 1.2 µM	40 nM	(46)
Au/Cyst/PAMAM/GNPs	1×10^{-5} - 2.5×10^{-3}	2 µM	(47)
GC/[bmim][PF6]-MWCNT /CAT	5-1700 nM	0.25 nM	(48)
GC/NiO-MWCNT/CAT	19–170 nM	2.4 nM	(49)

CONCLUSION

Catalase can be strongly adsorbed onto the surface of GC electrode to electrode form a stable, approximate Nafion-CdTe/CAT film through casting. Due to the promoting effort of CdTe quantum dots, the direct electron transfer between catalase and electrode was achieved. Based on this, the impedimetric measurement can be used as a basis for H_2O_2 detection. A wide linear relation was found between 1/Rct and H_2O_2 concentration. Since the DC potential applied in the EIS measurement are less cathodic than that applied in amperometric measurements, and more accurate data can be obtained using impedimetric biosensors. Thus, the instinctive problems due to high over potential applied in amperometric measurements can be resolve in impedimetric method. The observed high sensitivity and much lower limit of detection of the proposed nano-biosensor, relative to the reported amperometric sensors in the literature, clearly revealed that the nano-composite used not only resulted in facilitated communication between catalase and the modified electrode, but also provided a proper microenvironment for enzyme-substrate interaction.

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