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Research Article

Influences of Mg Doping on the Electrochemical Performance of TiO_2 Nanodots Based Biosensor Electrodes

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Electrochemical biosensors are essential for health monitors to help in diagnosis and detection of diseases. Enzyme adsorptions on biosensor electrodes and direct electron transfer between them have been recognized as key factors to affect biosensor performance. TiO_2 has a good protein adsorption ability and facilitates having more enzyme adsorption and better electron transfer. In this work, Mg ions are introduced into TiO_2 nanodots in order to further improve electrode performance because Mg ions are considered to have good affinity with proteins or enzymes. Mg doped TiO_2 nanodots on Ti substrates were prepared by spin-coating and calcining. The effects of Mg doping on the nanodots morphology and performance of the electrodes were investigated. The density and size of TiO_2 nanodots were obviously changed with Mg doping. The sensitivity of 2% Mg doped TiO_2 nanodots based biosensor electrode increased to 1377.64 from 897.8 μA mM^{-1} cm^{-2} and its K_M^{app} decreases to 0.83 from 1.27 mM, implying that the enzyme achieves higher catalytic efficiency due to better affinity of the enzyme with the Mg doped TiO_2. The present work could provide an alternative to improve biosensor performances.

1. Introduction

Electrochemical biosensors are widely investigated and applied in areas related to health monitors, environmental areas, and pharmaceutical and industrial fields [1–5]. Among them, hydrogen peroxide (H_2O_2) biosensors attract more and more attention because H_2O_2 is involved in most of biological events and intercellular pathways and is the by-product of oxidases such as cholesterol oxidase, lactate oxidase, and glutamate oxidase. Among various techniques, the electrochemical approach shows many advantages such as high sensitivity, low cost, rapid response, and simplicity [6].

In electrochemical sensors, direct electron transfer between the enzymes and electrodes has been identified as one of the key factors to determine their performance [7]. Since the direct electron transfer between the enzyme and the bare electrode is very difficult to take place [8], a mediator is usually required to establish a bridge between enzyme and electrode; thus, proper design of electrodes is an effective way to control the direct electron transfer [9]. TiO_2 is a wide band semiconductor with good chemical stability and biocompatibility, and it is reported that the modified electrodes with nanostructured mediator could accelerate the direct electron transfer rate [10, 11]. Hence, nanostructured TiO_2 with high surface area and high surface activity should be a good mediator with a strong protein adsorption ability that favors the electron transfer between enzymes and electrode [12]. There are many studies for doped TiO_2 with several approaches in order to modify and enhance the efficiency of the TiO_2 in various applications [13–17]. For biosensor applications, the doped TiO_2 provides more effective interaction area and more feasible electron transfer interface to support amperometric response of the electrode [18]. Mg ion has been doped in TiO_2 and it showed that the ion could improve the efficiency of TiO_2 in the medical applications [19] due to good affinity of Mg ions with proteins.

Based on our previous work on TiO_2 nanodots based biosensor electrode [20], in this work, we adopted Mg doping and attempted to improve performances of TiO_2 nanodots based H_2O_2 biosensor electrodes through strengthening

Table 1: Concentrations of Mg, TBOT, and PVP in the precursor sols for different TiO_2 nanodots/Ti samples*.

Sample name	Mg concentration (molar ratio)	TBOT (mol/L)	PVP (g/L)	Reference
Mg-TND-0	0%	0.25	45	[20]
Mg-TND-1	1%	0.25	45	This work
Mg-TND-2	2%	0.25	45	This work
Mg-TND-3	3%	0.25	45	This work
Mg-TND-4	4%	0.25	45	This work
Mg-TND-5	5%	0.25	45	This work
Mg-TND-6	6%	0.25	45	This work

*Note: Mg-TND is Mg-doped into TiO_2 nanodots/Ti substrate.

direct electron transfer with the aid of good affinity of Mg doped TiO_2 nanodots with the enzyme. The influences of Mg doping on TiO_2 nanodots and performance of the resulting biosensors were characterized and discussed.

2. Experimental Design

2.1. Preparation and Characterization of Mg Doped TiO_2 Nanodots Films on Ti Substrate. Titanium (Ti) foils with purity of 99.99% and thickness of 0.1 mm were used. The foils were cut into small substrates with dimensions of (2 × 1) cm and then ultrasonically cleaned in ethanol, deionized water, and acetone (1 : 1 : 1). After rinsing with deionized water and ethanol, the substrates were dried under room temperature. A phase-separation-induced self-assembly approach was used to prepare TiO_2 nanodots on Ti substrate [21]. Briefly, the precursor sol was prepared with ethanol solution of magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$, Hushi Chemical Reagent, AR), acetylacetone (AcAc, Lingfeng Chemical Reagent, AR, >99%), polyvinyl pyrrolidone (PVP, K30, Sinopharm Chemical Reagent, AR, >99%), deionized water, and titanium tetrabutoxide (TBOT, Sinopharm Chemical Reagent, CP, >98%). The molar ratio of H_2O : TBOT : AcAc was 1 : 1 : 0.3. Table 1 shows the concentrations of Mg, TBOT, and PVP in the precursor sols for different samples. The homogeneous precursor sol was spin-coated with 30 μL onto the Ti substrates at 7500 rpm for 50 s. Then the samples were calcinated in the air at 500°C for 90 min. A field-emission scanning electron microscope (FESEM) (Hitachi, S-4800) was used to observe the morphology of TiO_2 nanodots film.

2.2. Preparation of H_2O_2 Biosensors. Horseradish peroxidase enzyme (HRP) was selected because HRP is available with high sensitivity and purity, and it was used in the fabrication of biosensors for accurate and reliable determination of H_2O_2 [20, 22–25]. HRP was purchased from Aladdin Reagent (250 U mg^{-1}) and stored at 4°C. Nafion (5 wt%) was bought from Sigma-Aldrich, and 0.5 wt% Nafion solution was prepared to be used for immobilizing HRP enzyme and was stored in dark place at room temperature. 0.1 M PBS was prepared and it consisted of NaH_2PO_4, Na_2HPO_4, NaCl, and H_2O. The HCl and NaOH were used to adjust the pH of the PBS. The PBS solution was deoxygenated by bubbling pure N_2 gas for 30 min prior to use. The solution of H_2O_2 (hydrogen peroxide 30%, AR) was freshly prepared. The other chemicals employed in our experiments were of analytical grade and were used as supplied. The distilled deionized water was used for solutions.

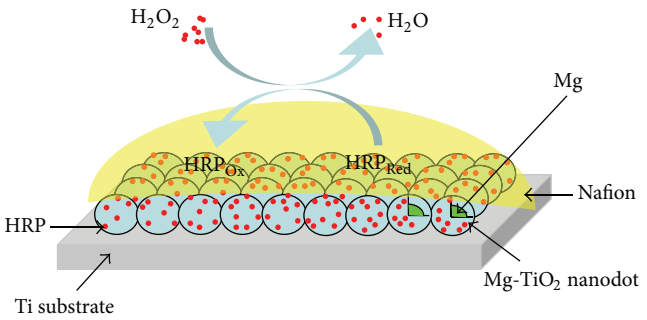


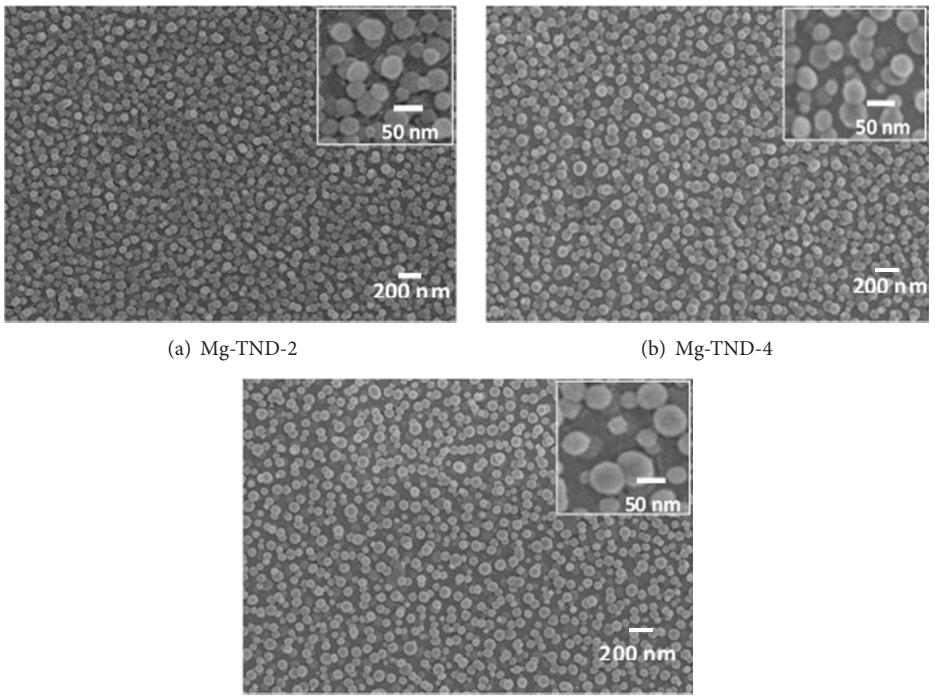
Figure 1: Schematic representation of the hydrogen peroxide biosensor fabricated on Nafion/HRP/Mg-TND/Ti.

Mg doped into TiO_2 nanodots film was sealed with epoxy except 2.5 × 2.5 mm^2; it was left as measuring area. The physical adsorption method was used to immobilize the HRP enzyme on the electrode surface, where 6 μL HRP solution was dropped on the electrode surface and left to dry at room temperature. The HRP solution was prepared by dissolving HRP in 0.01 M PBS (phosphate buffer solution with pH = 7.4 [26, 27]) in order to obtain 0.01 g mL^{-1} solution. Finally, 4 μL of 0.5 wt% Nafion solution was dropped onto the biosensor surface to protect the enzyme and make the biosensor be biocompatible, because Nafion has been used as an immobilization matrix for the enzyme and to keep the stability of the biosensor for long term [28], and then left it to dry at room temperature (Figure 1). The Nafion/HRP/Mg-TND/Ti electrodes were washed after storing at 4°C for 1 day [26, 27, 29]. The modified Nafion/HRP/Mg-TND/Ti electrodes were stored when not being in use at 4°C.

2.3. H_2O_2 Biosensors Characterization. The modified electrodes were tested by using CHI 660D electrochemical workstation. 0.1 M phosphate buffer solutions were prepared with various pH (5.0, 6.0, 6.5, 7.0, 7.4, and 8.0) in order to select the optimum pH value of the PBS that can show high performance for the biosensor. The performance of the present biosensor electrodes with pH response was shown to be highest at pH 7.0; therefore, pH value of BPS was set at 7.0 for

Table 2: Average diameters, densities, and specific area of TiO_2 nanodots with various Mg doped concentrations.

Sample name	Average diameter (nm)	Density ($\times 10^{10}$ cm^{-2})	Specific area of nanodots ($\times 10^{14}$ nm^2/cm^2)	Reference
Mg-TND-0	134	2.22	12.523	[20]
Mg-TND-2	139	2.12	12.868	This work
Mg-TND-4	145	1.82	12.021	This work
Mg-TND-6	149	1.71	11.926	This work



(a) Mg-TND-2 (b) Mg-TND-4

(c) Mg-TND-6

Figure 2: SEM images of TiO_2 nanodots/Ti substrates with various Mg doped concentrations with different morphologies.

measuring the electrode performances. The cyclic voltammetry techniques were used to test the modified electrodes in PBS 20 mL (PBS, 0.1 M at 25°C and purged with pure nitrogen for 30 min in order to remove the oxygen) and cycled by applying a voltage in the range between −0.2 V and −0.8 V. The amperometric technique was carried out on the biosensors, whereas various applied potential values were applied from −0.4 V to −0.9 V in order to find the optimum applied voltage for the biosensor that give high current response and therefore −0.8 V was selected as optimum applied potential value.

3. Results and Discussion

3.1. Microstructures of Mg Doped TiO_2 Nanodots Based Electrodes. After Mg was doped, the resulting films had similar nanodot morphology; it indicates that the addition of Mg ions in the 0~6 mol% range could not have influence on Marangoni effect induced phase separation during spin-coating, which induces nanodot morphology [21]. However, Mg doping was shown to have changes in density and size of TiO_2 nanodots on Ti (Figure 2). Compared with undoped TiO_2 nanodots, the density of TiO_2 nanodots was inversely proportional to Mg concentration, and the size of TiO_2 nanodots was proportional to the Mg concentration (Table 2). This change could be attributed to the fact that the spherical phase separation in spun thin liquid layer is influenced by chloride anions from Mg precursor. For observation of an individual TiO_2 nanodot under TEM, it was found that the distribution of Mg element in TiO_2 nanodots was homogenous (Figure 3), which favors adsorbing proteins or enzymes.

3.2. Electrochemical Behaviors of Mg Doped TiO_2 Nanodots Based Biosensor Electrodes. Figure 4 shows the cyclic voltammetric curve of Mg doped TiO_2 nanodots based biosensor electrode, indicating that a typical reaction mechanism of hydrogen peroxide biosensor occurred as follows [30, 31]:

$$\begin{aligned} HRP_{Red} + H_2O_2 &\longrightarrow HRP_{Ox} + H_2O \\ HRP_{Ox} + 2e^- + 2H^+ &\longrightarrow HRP_{Red} + H_2O \end{aligned} \quad (1)$$

For the biosensor electrodes with different Mg doping amount, all had a small pair of redox peaks, and Nafion/HRP/

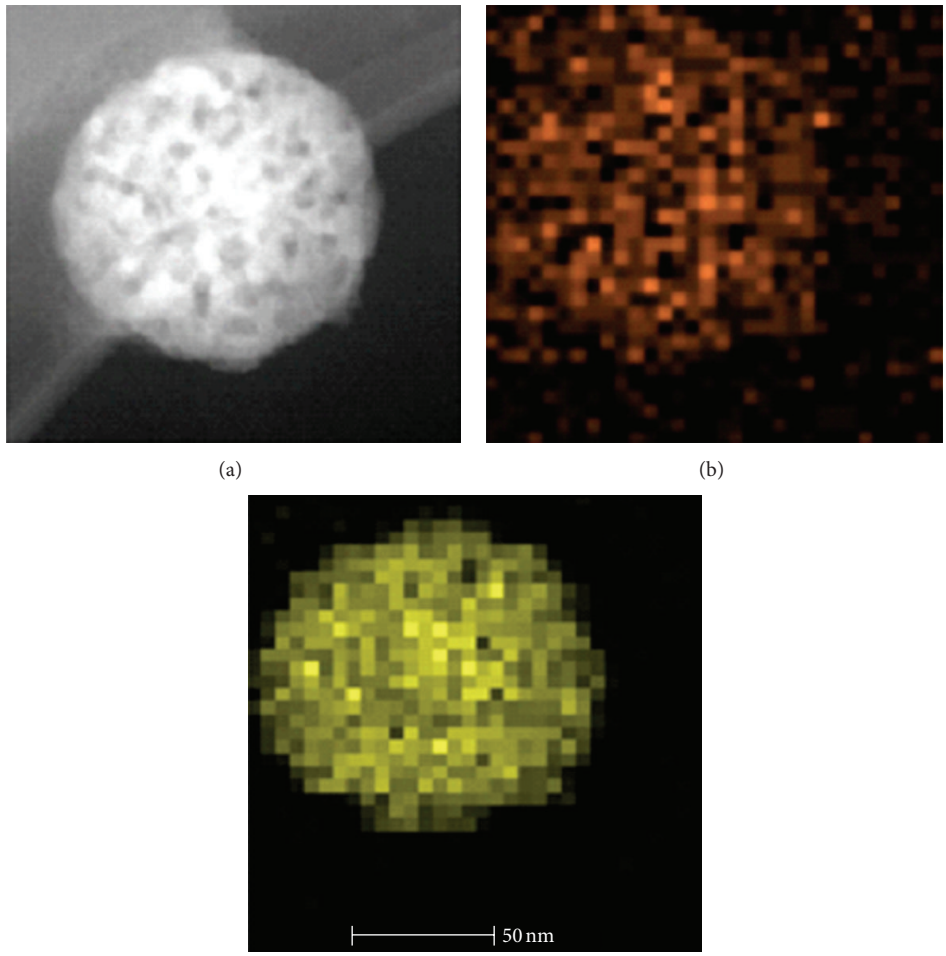


Figure 3: TEM images; (a) TiO_2 nanodots film with Mg doped, (b) Mg element mapping, and (c) Ti element mapping.

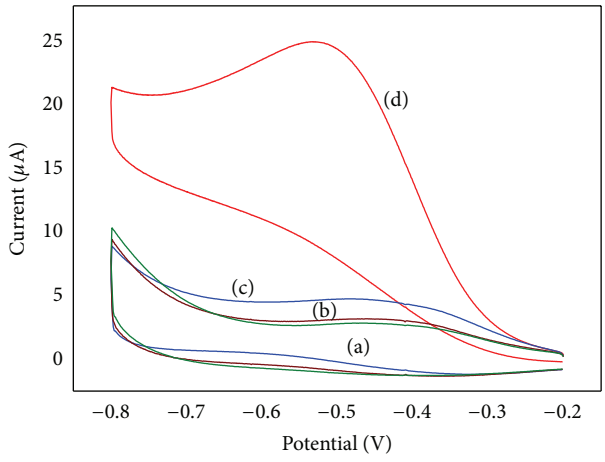


Figure 4: Cyclic voltammetry of (a) Nafion/HRP/Mg-TND-6 electrode, (b) Nafion/HRP/Mg-TND-4 electrode, (c) Nafion/HRP/Mg-TND-2 electrode in 0.1 M PBS (pH = 7.0) and 0.1 $V \cdot s^{-1}$ scan rate, and (d) Nafion/HRP/TND-3 electrode in 0.1 M PBS (pH = 7.0) with 100 μM H_2O_2 and 0.1 $V \cdot s^{-1}$ scan rate.

Mg-TND-4/Ti and Nafion/HRP/TND-6/Ti biosensor electrodes (curves (a) and (b)) had smaller cathodic peak potential than Nafion/HRP/Mg-TND-2/Ti biosensor electrode (curve (c)), implying that Nafion/HRP/Mg-TND-2/Ti improved well the electrochemical response of HRP enzyme [32]. The better voltammetric response could result from better establishment in electron transfer between the electrode surface and HRP in the Nafion/HRP/Mg-TND-2/Ti electrode.

The anodic peak potential E_{pa} and the cathodic peak potential E_{pc} of Nafion/HRP/Mg-TND-2/Ti were found at −0.335 V and −0.473 V, respectively (curve (c)). The peak to peak separation ΔE_p was found to be 0.138 V. When 100 μM H_2O_2 into 0.1 M PBS was added, the Nafion/HRP/TND-3/Ti biosensor electrode was shown to have a good response (curve (d)), indicating that a strong electrocatalytic activity appears in the reduction reaction of H_2O_2. Also the direct electron transfer rate in Nafion/HRP/Mg-TND-2/Ti is shown to be faster than that of the undoped biosensor electrode. This could be attributed to good function of the immobilized HRP on Mg doped TiO_2 nanodots.

Table 3: The specific parameters of the four biosensor electrodes.

Electrode	LOD (μM)	Sensitivity (μA·mM^{-1}·cm^{-2})	Reference
Nafion/HRP/TND-3/Ti	0.26	897.8	[20]
Nafion/HRP/Mg-TND-2/Ti	0.027	1377.64	
Nafion/HRP/Mg-TND-4/Ti	0.031	811.12	
Nafion/HRP/Mg-TND-6/Ti	0.04	643.32	

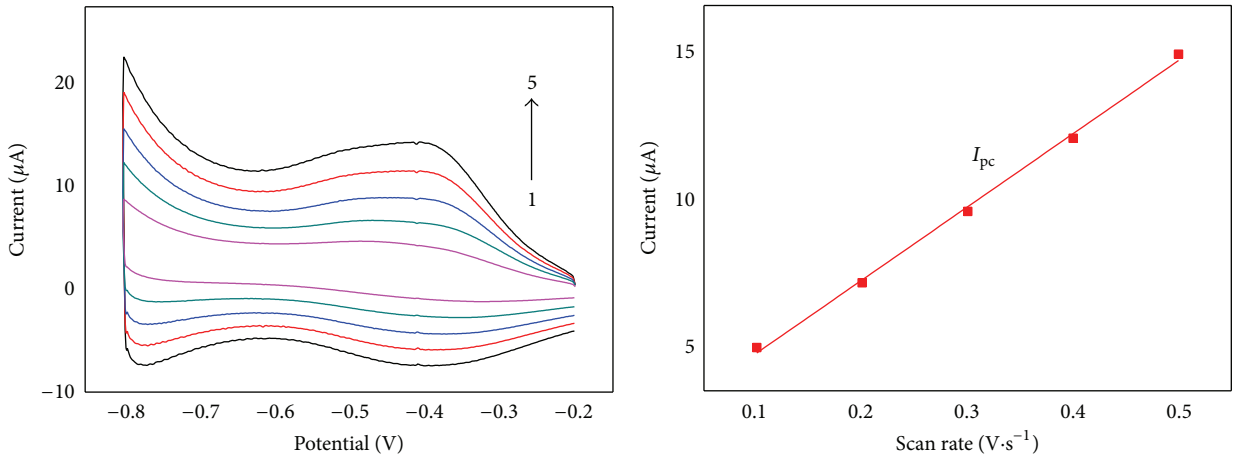


Figure 5: (a) Cyclic voltammetry of modified biosensor Nafion/HRP/Mg-TND-2 in PBS (0.1 M, pH = 7.0) with various scan rates. (b) Plot reduction peaks current versus scan rates.

Moreover, the CV curves of Nafion/HRP/Mg-TND-2/Ti biosensor electrode were obtained with various scan rates. It was found that the reduction peak current increased linearly with increasing scan rate (from 0.1 to 0.5 V·s^{-1}) in 0.1 M PBS with pH = 7.0 (Figure 5(b)). The increase of cathodic peaks is ascribed to occurrence of an enhanced electrochemical reaction of the biosensor electrode and indicates that the reaction is surface-controlled and the direct electron transfer on the biosensor electrode takes place well [33, 34]. Hence, Mg doping could intensify the electrochemical response of TiO_2 nanodots based biosensor electrode.

3.3. Performance and Stability of Mg Doped TiO_2 Nanodots Based Biosensor Electrodes. Figure 6 shows the amperometric responses of the Nafion/HRP/Mg-TND/Ti biosensor electrodes with successive additions of 40 μM H_2O_2. The sensitivity and detection limit of the electrodes were calculated and listed in Table 3; Nafion/HRP/Mg-TND-2/Ti electrode was demonstrated to be the best among them. For Nafion/HRP/Mg-TND-2/Ti biosensor electrode, the reduction current with addition of H_2O_2 increased rapidly and reached 95% of steady-state current within 3 s (Figure 6(a)). A linear relationship between current and H_2O_2 concentration was found in Figure 6(b), and the linear range of the current was from 6 μM to 640 μM with coefficient coloration $R = 0.9996$ ($n = 17$). Moreover, Nafion/HRP/Mg-TND-2/Ti electrode had a limit of detection (LOD) of 0.027 μM (evaluated at a signal-to-noise ratio of 3, according to [35]) and the sensitivity of 1377.64 μA mM^{-1} cm^{-2} which is 1.53-fold for the undoped electrode.

Although the sensitivities of the corresponding biosensor increased with the specific area of the nanodots films, the contribution of Mg ions is obvious. The specific area of Nafion/HRP/Mg-2-TND/Ti is just 1.09-fold of Nafion/HRP/TND-3/Ti electrodes (Table 2) but big difference in sensitivity was observed (Table 3). Hence, it is suggested that both of large specific area and proper Mg doping promote enzyme adsorption and function. The biosensor performance of Nafion/HRP/Mg-2-TND/Ti electrode is also better than those of HRP/TiO_2 microsphere/Nafion/Ti [27], Nafion/HRP/Au-TiO_2 nanoparticle/GCE [32], Nafion/HRP/TN-3/ITO [26], and HRP/Fe_3O_4/m-silica nanoparticle/SPE [29]. The stability of the Nafion/HRP/Mg-TND-2/Ti biosensor was investigated by amperometric technique, whereas the biosensor is stored for 12 days at 4°C. The current response of the biosensor was measured with the same conditions and it was found to be more than 89% retaining of the activity and indicates that the biosensor is stable for biosensor applications.

Moreover, the apparent Michaelis-Menten constant (K_M^{app}) was calculated from the Lineweaver-Burk equation because K_M^{app} is one of the key measureable parameters related to working status of enzymes [36]:

$$\frac{1}{I_{ss}} = \frac{1}{I_{max}} + \frac{K_M^{app}}{I_{max}C}, \quad (2)$$

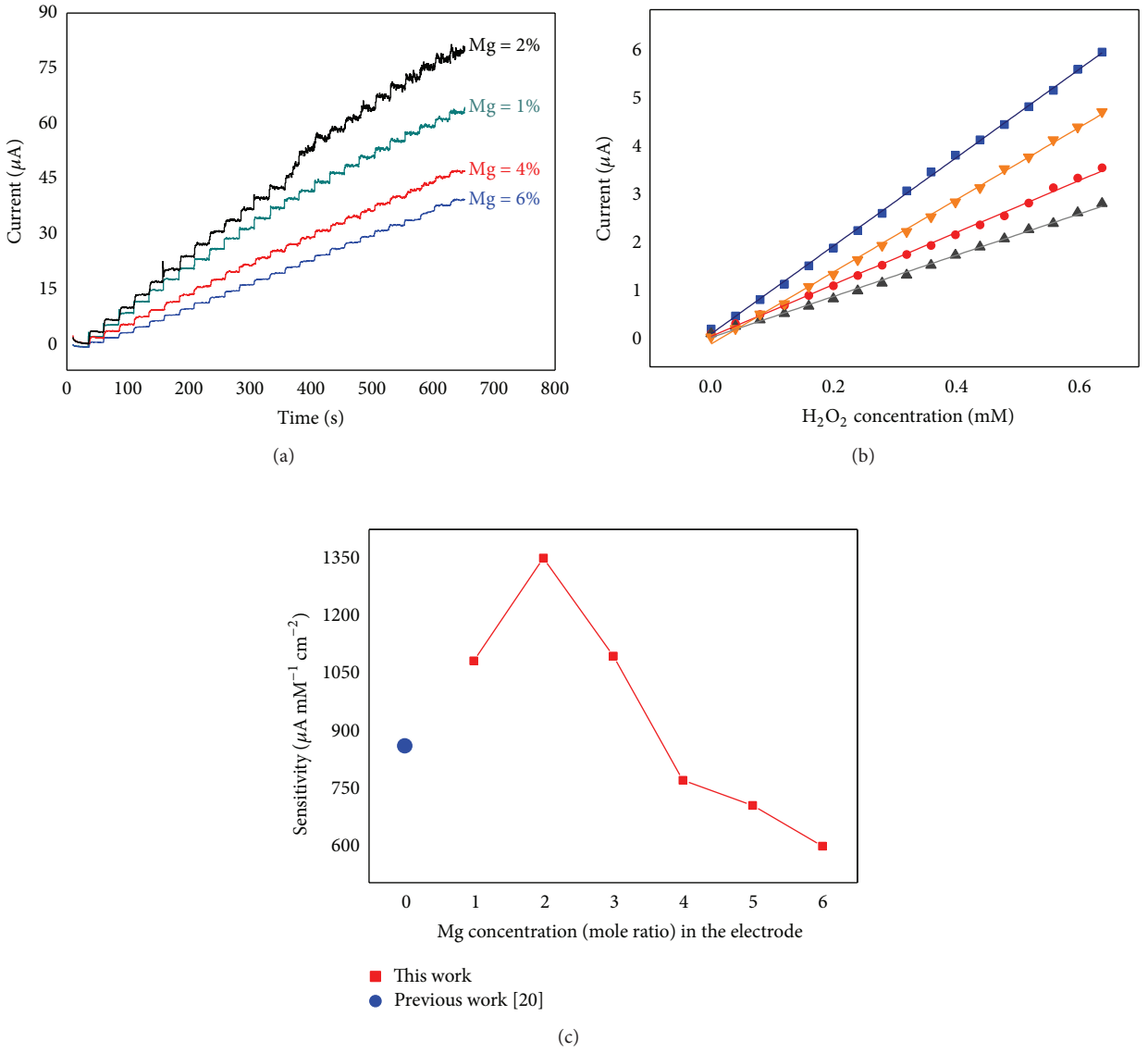


Figure 6: (a) Typical amperometric technique (I-t) curve of different modified electrodes (with different Mg concentrations (mole ratio): 1%, 2%, 4%, and 6%) after adding H_2O_2 in stirred solution of 0.1 M PBS; (b) calibration plot of response current versus H_2O_2 concentration of different modified electrodes (with different Mg concentrations (mole ratio); 1%, 2%, 4%, and 6%); (c) the sensitivity of different electrodes.

where I_{ss} is the steady-state current after the addition of substrate, I_{max} is the maximum current measured under saturated bulk solution condition, and C is the bulk concentration. The K_M^{app} of Nafion/HRP/Mg-2-TND/Ti electrode was calculated to be 0.83 mM and to be smaller than Nafion/HRP/TND/Ti electrode (1.27 mM). This represents the fact that the enzyme achieves higher catalytic efficiency at low H_2O_2 concentration due to better affinity of the enzyme with Mg doped TiO_2.

4. Conclusions

Mg doped TiO_2 nanodots based electrode was prepared by sol-gel spin-coating, followed by calcination. It was found that 2% Mg doping TiO_2 nanodots electrode has better electrochemical response and biosensing performance than undoped electrode, with the sensitivity of 1377.64 μA mM^{-1} cm^{-2}, which is 1.53-fold. The reason is that Mg doping intensifies direct electrode transfer on the electrode due to good affinity of Mg doped TiO_2 with the working enzyme as well as increase of specific area of the nanodots. Such doping approach could provide an effective way to improve performance of the electrode of amperometric biosensors through chemical modifications.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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