Electrochemical synthesis and characterization of TiO$_2$ nanoparticles and their use as a platform for flavin adenine dinucleotide immobilization and efficient electrocatalysis

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Abstract

Here, we report the electrochemical synthesis of TiO$_2$ nanoparticles (NPs) using the potentiostat method. Synthesized particles have been characterized by using x-ray diffraction (XRD) studies, atomic force microscopy (AFM) and scanning electron microscopy (SEM). The results revealed that the TiO$_2$ film produced was mainly composed of rutile and that the particles are of a size in the range of 100 ± 50 nm. TiO$_2$ NPs were used for the modification of a screen printed carbon electrode (SPE). The resulting TiO$_2$ film coated SPE was used to immobilize flavin adenine dinucleotide (FAD). The flavin enzyme firmly attached onto the metal oxide surface and this modified electrode showed promising electrocatalytic activities towards the reduction of hydrogen peroxide (H$_2$O$_2$) in physiological conditions. The immobilized FAD displayed a pair of redox peaks with a formal potential of $-0.42$ V in pH 7.0 oxygen-free phosphate buffers at a scan rate of 50 mV s$^{-1}$. The FAD in the nanostructured TiO$_2$ film retained its bioactivity and exhibited excellent electrocatalytic response to the reduction of H$_2$O$_2$, based on which a mediated biosensor for H$_2$O$_2$ was achieved. The linear range for the determination of H$_2$O$_2$ was from $0.15 \times 10^{-6}$ to $3.0 \times 10^{-3}$ M with the detection limit of $0.1 \times 10^{-6}$ M at a signal-to-noise ratio of 3. The stability and repeatability of the biosensor is also discussed. (Some figures in this article are in colour only in the electronic version)

1. Introduction

Hydrogen peroxide (H$_2$O$_2$) is used as an oxidant, disinfectant and bleaching agent in various industrial and household applications [1–3]. The broad occurrence and application of H$_2$O$_2$ therefore require the use of suitable analytical methods for its determination from trace concentration levels up to the levels typically found in food, pharmaceutical, clinical, industrial and environmental samples. H$_2$O$_2$ also plays a key role as the product of the enzymatic reaction in coupled enzyme systems [4]. Owing to its importance, several analytical techniques have been employed for its determination, such as titrimetry [5], spectrometry [6], chemiluminescence [7, 8] and electroanalytical methods [9–14]. Among these methods, electroanalytical methods based on the enzyme modified electrodes have several advantages such as low detection limit, less interference, low cost, fast response and being suitable for field analysis.

The flavins are an important class of biochemical compounds which are found in both plants and animals. Due to their importance, most of the flavins have been the subject of...
In the present paper, we report a disposable amperometric biosensor for the quantification of H$_2$O$_2$ in physiological conditions. First, TiO$_2$ NPs were electrochemically synthesized on an indium tin oxide coated electrode; they were characterized by XRD, AFM and SEM. The TiO$_2$ NPs were used to modify a screen printed carbon electrode (SPE), and the resulting TiO$_2$NPs/SPE was used to immobilize FAD enzyme using consecutive cyclic voltammetry (CV). Electrochemical synthesis of TiO$_2$ was carried out on the ITO electrode. Hydrodynamic voltammetric studies on the dioxygen reduction reaction and amperometry measurements of H$_2$O$_2$ were performed on a Bi-potentiostat Model CHI750A (TX, USA) having an analytical rotator model AFMSRK with MSRX speed control (Rotatable disk electrode PINE Instruments, USA). A model S-3000H scanning electron microscope (Hitachi Scientific Instruments, London, UK) was used for surface image measurements. The AFM images were recorded with a multimode scanning probe microscope system operated in tapping mode using a Being Nano-Instruments CSPM-4000 (Ben Yuan Ltd, Beijing, China). Electrochemical measurements were performed with a CHI750A Electrochemical Work Station (CH Instrument Inc., USA). Disposable screen printed carbon electrodes were purchased from Zensor R&D (Taichung, Taiwan) and indium tin oxide (ITO) coated glass electrodes were purchased from Merck Display Technologies, Ltd (Darmstadt, Germany). The ITO thickness and resistance were 30 ± 10 nm and 80 Ω, respectively. The size of the glass was 300 mm × 350 mm × 0.7 mm. The ITO and SPE were used as working electrodes. ITO substrates were cleaned by using detergent, diluted nitric acid and then finally rinsed with distilled water. Platinum wire was used as the counter electrode. All the cell potentials were measured with respect to an Ag/AgCl [KCl (sat)] reference electrode.

2.2. Electrochemical synthesis of TiO$_2$

Cathodic electroosynthesis of TiO$_2$ NPs was carried out on the ITO electrode from a bath solution containing 0.02 M TiOSO$_4$, 0.03 M H$_2$O$_2$, 0.05 M HNO$_3$ and 0.05–0.25 M KNO$_3$ (pH 1.4). The deposition was performed at room temperature (25 ± 2°C) under potentiostatic conditions (−2.0 V versus Ag/AgCl). This led to the formation of a white color gel film on the electrode surface. Each deposition was carried out for 30 min. For the preparation of multiple TiO$_2$ layers, the electrosynthesis was repeated three or four times, with drying steps at 150°C in between, after which there was a final annealing step at 400°C for 1 h to obtain crystalline TiO$_2$ film. The substrates were weighed prior to coating and after annealing to determine the amount of deposited TiO$_2$. Nearly 20% mass reduction was observed after heat treatment at 400°C for 1 h, due to the elimination of water from the film [30]. Thereafter, crystalline TiO$_2$ particles were scratched from the ITO surface and collected in 10 ml brown colored vial and used later for modification of the SPE. Cathodic electrodeposition of TiO$_2$ film from TiOSO$_4$ + H$_2$O$_2$ + HNO$_3$ + KNO$_3$ (pH 1.4) solutions involves the indirect deposition of a gel of hydrous titanium oxo-hydrides (equation (3)), resulting from the reaction of titanium peroxo-sulfate (equation (2)) with the hydroxide ions produced by nitrate electrochemical reduction [31, 32].

\[
\text{NO}_3^- + \text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{NO}_2^- + 2\text{OH}^- \tag{1}
\]

\[
\text{TiOSO}_4 + \text{H}_2\text{O}_2 \rightarrow \text{Ti(O}_2\text{)SO}_4 + \text{H}_2\text{O} \tag{2}
\]

\[
\text{Ti(O}_2\text{)SO}_4 + 2\text{OH}^- + (x + 1)\text{H}_2\text{O} \rightarrow \text{TiO(OH)}_{2-x}\text{H}_2\text{O}_2 + \text{SO}_4^{2-} \tag{3}
\]
Annealing of the gel at 400°C for 1 h results in the formation of crystalline TiO₂ particles (proved by XRD analysis to be rutile).

\[
\text{TiO(OH)}_2 \cdot x\text{H}_2\text{O} \rightarrow \text{TiO}_2 + (x + 1)\text{H}_2\text{O}. \tag{4}
\]

2.3. Preparation of modified electrodes

5 mg of TiO₂ NPs was added into 10 ml double-distilled water and then ultrasonicated for 10 min to create a suspension with a concentration of 0.5 mg ml⁻¹. After being diluted by five times, 10 µl of TiO₂ NP suspension was spread evenly onto the surface of the SPE, which was then dried for 6 h in the absence of light. Finally, the modified electrode was thoroughly rinsed with double-distilled water. The TiO₂NPs/SPE was cycled in 0.1 M H₂SO₄ solution containing 2 × 10⁻⁴ M FAD between the potential ranges of 0.2 to −0.4 V for 40 cycles. Afterward, the electrode was thoroughly rinsed with double-distilled water and then dried at 4°C for 1 h in the absence of light. When not in use, the electrode was stored in aqueous solution of 0.1 M phosphate buffer solution (pH 7.0) at 4°C. It is denoted as FAD/TiO₂NPs/SPE, and it was used for further studies. For comparison, a TiO₂NPs coated electrode was prepared and used for further investigation.

3. Results and discussions

3.1. Surface characterizations

AFM (tapping mode) was used to record the topography of the modified electrodes. In this mode, the probe cantilever is oscillated at or near its resonant frequency. The surface morphologies of the TiO₂NPs and FAD/TiO₂NPs coated electrodes exhibit notable features. Figures 1(a) and (b) show 3D AFM images (20 µm × 20 µm) of the TiO₂NPs and FAD/TiO₂NPs films. The average surface roughnesses and peak-to-peak height values are 16, 19 nm and 132, 203 nm for TiO₂NPs and FAD/TiO₂NPs, respectively. The FAD/TiO₂NPs film thickness was 61 nm more than that of the TiO₂NPs film; this difference arose due to the adsorption of FAD films on the oxide surface. The average particle sizes of TiO₂ are found to be in the range of 100 ± 50 nm. Figures 1(c) and (d) show SEM images of the TiO₂NPs and FAD/TiO₂NPs films. The spherical particle aggregates cover the surface (figure 1(c)). The large amount of TiO₂ in the SEM image (figure 1(d)) can be explained by the migration of particles at the surface and adsorption of FAD films on the oxide surface. Figure 2 shows the x-ray diffraction patterns of TiO₂ particles. The experimental spacing were compared with those reported for rutile (110) (2θ of 27.45°) and anatase (101) (25.24°) to identify the particle structure [33]. The XRD results revealed that the synthesized particles are mainly composed of rutile.

3.2. Electrochemical impedance studies

CV and electrochemical impedance spectroscopy (EIS) were used for monitoring each step of SPE modification [34]. The measurements were done in phosphate buffer solution (0.1 M NaH₂PO₄ + 0.1 M KCl; pH 7.0) in the presence of 1 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆]. As expected, K₃[Fe(CN)₆]/K₄[Fe(CN)₆] shows reversible behavior on a bare SPE (figure 3, curve a). After the attachment of TiO₂ NPs and FAD, the shape of the cyclic voltammograms changes dramatically. TiO₂NPs and TiO₂NPs/FAD films on the surface of the SPE restrict the electrode conduction (figure 3 curves b and c).

The complex impedance is displayed as the sum of the real (Z_re) and imaginary (Z_im) components. The bare SPE exhibits
Figure 2. X-ray diffraction patterns of TiO$_2$ particles.

Figure 3. Cyclic voltammograms recorded in 0.1 M PBS containing 1 mM K$_3$[Fe(CN)$_6$]$_{3-}$/Fe(CN)$_3$$_6^-$ and 0.1 M KCl: (a) the bare SPE, (b) the TiO$_2$NPs/SPE, and (c) the FAD/TiO$_2$NPs/SPE.

Figure 4. The Nyquist plots for (1) the bare SPE, (2) the TiO$_2$NPs/SPE and (3) the FAD/TiO$_2$NPs/SPE in the presence of 1 mM Fe(CN)$_6^{3-}$/Fe(CN)$_3^-$ by applying an AC voltage with 50 mV amplitude in a frequency range from 100 mHz to 1000 kHz. The electrode potential was 0.18 V versus Ag/AgCl. The inset is the Randle’s equivalent circuit for FAD/TiO$_2$NPs/SPE.

Figure 5 shows the cyclic voltammograms of the FAD/TiO$_2$NPs/SPE in acidic solution (pH 1.5) at different scan rates. One reversible redox couple was observed at $E^0' - 0.12$ V versus Ag/AgCl electrode. Also, the electrochemical response of the FAD/TiO$_2$NPs/SPE is anticipated for a surface-confined redox couple, because the peak currents were directly proportional to the scan rate up to 200 mV s$^{-1}$ (figure 5), as predicted for a surface-confined process. The ratio of cathodic to anodic peak currents at various scan rates was almost constant. The peak-to-peak potential separation ($\Delta E_p$) is about 37 mV for FAD redox peaks at sweep rates below 100 mV s$^{-1}$, suggesting facile charge transfer kinetics over this range of sweep rate. The surface coverage concentration ($\Gamma_0$) of FAD was evaluated from the following equation:

$$\Gamma_0 = \frac{Q}{nFA}$$

where $A$ (=0.1963 cm$^2$) is the area of the SPE, $n$ (=2) the number of electrons per reactant molecule, $Q$ the charge obtained by integrating the anodic peak at low voltage scan rate (20 mV s$^{-1}$), and $F$ is the Faraday constant. We assume that all of the immobilized redox centers are electroactive on the voltammetry timescale. In the present case, the calculated value of $\Gamma_0$ was 1.5697 × 10$^{-10}$ mol cm$^{-2}$. The formal potential of the FAD redox peak was pH dependent (data not shown). A plot of $E^0'$ versus pH gives a straight line from pH 1 to 13 with a slope of $-52$ mV/pH, which is very close to the anticipated Nernstian value of $-59$ mV for a two-electron–two-proton process of FAD [37–39] (scheme 1).
3.4. Electrocatalysis and amperometric detection of H$_2$O$_2$

To investigate the electrocatalytic activity of the FAD/TiO$_2$ NPs/SPE, electrochemical catalytic reduction H$_2$O$_2$ at the FAD/TiO$_2$NPs/SPE was investigated by CV (figure 6). There was no peak observed at bare the SPE in the presence of H$_2$O$_2$ in the potential range of $-0.1$ to $-0.7$ V, suggesting that the SPE was inactive to the direct reduction of H$_2$O$_2$ (curve c). However, at the FAD/TiO$_2$NPs/SPE, the reduction peak current at about $-0.45$ V was greatly enhanced in the presence of H$_2$O$_2$ corresponding with the decrease of the oxidation peak current, suggesting a typical electrocatalytic reduction process of H$_2$O$_2$. The reduction peak current increased with the concentration of H$_2$O$_2$ in the solution (curve b). In the absence of H$_2$O$_2$ a reversible redox peak was observed (curve a). The above results indicated that mediated reduction of H$_2$O$_2$ takes place at the FAD/TiO$_2$NPs/SPE, as described in equations (6), and (7).

The amperometric response of the FAD/TiO$_2$NPs/SPE to successive addition of different concentration of H$_2$O$_2$ at the working potential of $-0.45$ V was also investigated (figure 7). The inset in figure 7 demonstrates the linear relationship of the electrocatalytic current ($I_{cat}$) versus H$_2$O$_2$ concentration in the wide range of $0.15 \times 10^{-6}$–$3.0 \times 10^{-3}$ M. The detection limit was found to be $0.1 \times 10^{-6}$ M ($S/N=3$) and the sensitivity of the FAD/TiO$_2$NPs/SPE was 1.86 A M$^{-1}$.

$$\text{FAD} + 2\text{H}^+ + 2e^- \leftrightarrow \text{FADH}_2 \quad (6)$$

$$\text{FADH}_2 + \text{H}_2\text{O}_2 \rightarrow \text{FAD} + 2\text{H}_2\text{O} \quad (7)$$

3.5. Electrocatalysis of oxygen

A direct electrocatalytic reduction reaction of O$_2$ was studied using the FAD/TiO$_2$NPs/SPE. Figure 8(A) shows the cyclic voltammograms of the FAD/TiO$_2$NPs/SPE in 0.1 M PBS (pH 7.0) containing O$_2$ saturated solution. A large increase in the cathodic peak at about $-0.48$ V is observed in the presence of O$_2$, and the increase in the cathodic peak is accompanied by a decrease of the anodic peak, suggesting that FAD has mediated the electrocatalytic reduction of O$_2$ (curve b). The TiO$_2$NPs/SPE and bare SPE did not show a significant increase in the peak current and failed to reduce the overpotential required for the O$_2$ reduction reaction (curves c and d). Indeed, the FAD/TiO$_2$NPs/SPE reduced the overpotential about 340 mV. In nitrogen saturated solution a reversible redox peak was obtained (curve a).
Figure 7. The amperometric responses recorded using the FAD/TiO2NPs/SPE at an applied potential of –0.45 V versus Ag/AgCl to successive addition of 0.15 mM H2O2 in 0.1 M PBS (pH 7.0), rotation rate ~600 rpm. The inset figure shows the calibration plot.

Figure 8(B) represents a set of current–potential curves in an O2 saturated buffer solution (pH 7.0) at various angular velocities, \( \omega \), with a rotating glassy carbon disk electrode modified with FAD/TiO2NPs. Curve (a') is the response of the modified electrode in the absence of oxygen. The limiting current, \( I_l \), is defined as the difference between the currents on a modified electrode at the potential corresponding to the diffusion plateau in deaerated and O2 saturated solutions. The corresponding Koutecky–Levich plot (figure 8(B)) also shows a linear relationship between the currents on a modified electrode at the potential corresponding to the diffusion plateau in deaerated and O2 saturated solutions. The Koutecky–Levich plot in figure 8(B) is very close to the theoretically calculated line for a two-electron process (\( n = 2 \)) and exhibits a linear relationship between the limiting current and rotation rate (\( \omega \)) values. The corresponding Koutecky–Levich plot (figure 8(B)) also shows a linear relationship between the limiting current and rotation rate (\( \omega \)) values. The corresponding Koutecky–Levich plot (figure 8(B)) also shows a linear relationship between the limiting current and rotation rate (\( \omega \)) values. The corresponding Koutecky–Levich plot (figure 8(B)) also shows a linear relationship between the limiting current and rotation rate (\( \omega \)) values. The corresponding Koutecky–Levich plot (figure 8(B)) also shows a linear relationship between the limiting current and rotation rate (\( \omega \)) values. The corresponding Koutecky–Levich plot (figure 8(B)) also shows a linear relationship between the limiting current and rotation rate (\( \omega \)) values.

\[
\text{FADH}_2 + \text{O}_2 \rightarrow \text{FAD} + \text{H}_2\text{O}_2. \tag{8}
\]

3.6. Reproducibility and stability of the modified electrode

The reproducibility of the current response of the biosensor was examined by measuring the H2O2 concentration of 1.5 mM, and the relative standard deviation was 2.5 (\( n = 9 \)). It was indicated that the biosensor possessed good reproducibility. In addition, the catalytic current response for reduction of H2O2 at the FAD/TiO2NPs/SPE was tested in the solution containing 1.5 mM H2O2 before and after continuously stirring the buffer solution for 30 min. The response of the electrode signal had no significant change before and after stirring the solution; this test indicated that reproducible results can be obtained at the FAD/TiO2NPs/SPE. After those experiments, the sensor was kept in 0.1 M PBS at 4°C in order to maintain the activity of FAD. We used the biosensor to detect H2O2 three times every day, and the results showed that the catalytic current only decreased about 3.0% after a month. The above results indicated that the FAD/TiO2NPs/SPE has good stability and that the irreversible adsorption of FAD that occurred onto the TiO2 films may be due to the interaction between the phosphate moiety of FAD and the TiO2 layer [41, 42]. According to the earlier reports, phosphate groups have more affinity towards TiO2 particles; this was confirmed by UV–visible spectra and electrochemical studies [38, 43]. The phosphate groups of FAD may interact strongly with TiO2 films. Since the electrode preparation is simple, it can be easily prepared and is suitable for commercial purpose.
and electrocatalytic activity towards the reduction of H$_2$O$_2$ (sample B) (~3% H$_2$O$_2$) were analyzed. The commercial solutions were diluted 200 times in phosphate buffer solution; the diluted samples were analyzed with the FAD/TiO$_2$NPs/SPE using a chronoamperometric method. The results obtained are presented in Table 1. In our experiments, the concentration of H$_2$O$_2$ was calculated using the standard additions method. The relative standard deviation of each sample for five successive detections is less than 3.36%. In addition, the recovery ratio on the basis of this method was investigated, and the value is between 98.40 and 98.33%. The recovered ratio indicates that the determination of H$_2$O$_2$ using the FAD/TiO$_2$NPs/SPE is effective and can be applied for the detection of H$_2$O$_2$ in real samples.

### 3.7. Determination of H$_2$O$_2$ in real samples

To examine the real application of this modified electrode, measurements of H$_2$O$_2$ in commercially available antiseptic solutions (sample A) and soft contact lens cleaning solutions (sample B) (~3% H$_2$O$_2$) were analyzed. The commercial solutions were diluted 200 times in phosphate buffer solution; the diluted solutions were analyzed using the FAD/TiO$_2$NPs/SPE with a chronocoulometric method. The results obtained are presented in Table 1. In our experiments, the concentration of H$_2$O$_2$ was calculated using the standard additions method. The relative standard deviation of each sample for five successive detections is less than 3.36%. In addition, the recovery ratio on the basis of this method was investigated, and the value is between 98.40 and 98.33%. The recovered ratio indicates that the determination of H$_2$O$_2$ using the FAD/TiO$_2$NPs/SPE is effective and can be applied for the detection of H$_2$O$_2$ in real samples.

### 4. Conclusions

The electrochemical synthesis of TiO$_2$ NPs and their applications as a platform for FAD immobilization have been studied. The FAD/TiO$_2$NPs/SPE shows excellent stability and electrocatalytic activity towards the reduction of H$_2$O$_2$ and O$_2$ in physiological conditions. AFM, SEM and x-ray diffraction analyses revealed that the TiO$_2$NPs have covered the electrode surface, leading to the adsorption of FAD films. The electrocatalytic properties of the modified electrode were studied by using cyclic voltammetry, amperometry and RDE methods. The FAD/TiO$_2$NPs/SPE has been employed as a biosensor for the determination of H$_2$O$_2$ in the range from 0.15 x 10$^{-6}$ to 3.0 x 10$^{-3}$ M with a detection limit of 0.1 x 10$^{-6}$ M (S/N = 3).

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### References


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*Average value of five measurements.*