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Electrochemical Performance and Biosensor Application of TiO$_2$ Nanotube Arrays with Mesoporous Structures Constructed by Chemical Etching

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Abstract: Novel mesoporous TiO$_2$ nanotube arrays (TiO$_2$ NTAs) are synthesized by anodization method combined with chemical etching in HF solution, and the electrochemical performances are studied. Glucose oxidase (GOx) is immobilized on the mesoporous TiO$_2$ NTAs to achieve an efficient biosensor for amperometric detection of glucose. The morphology, structure, component and electrochemical performance of mesoporous TiO$_2$ NTAs are characterized by scanning electron microscope, high resolution transmission electron microscope, X-ray diffractometer, X-ray photoelectron spectrometer and electrochemical workstation, respectively. The influence of mesoporous structure on the electrochemical performance is discussed in detail by comparing the cyclic voltammograms and electrochemical impedance spectrum of TiO$_2$ and mesoporous TiO$_2$ NTAs in different conditions. High electrochemical active surface area and electron transfer rate play key roles on enhancing the electrochemical performance of mesoporous TiO$_2$ NTAs. When used as basis of biosensor, the amperometric response of glucose on GOx/TiO$_2$-0.5 NTAs electrode is linearly proportion to glucose concentration in the range from 0.1 to 6 mM with a sensitivity of 0.954 $\mu$A·mM$^{-1}$·cm$^{-2}$, which is 14.3 times that of un-etched GOx/TiO$_2$ NTAs.

Keywords: TiO$_2$ nanotube arrays; Mesoporous structure; Chemical etching; Electrochemistry; Biosensor

1 Introduction

TiO$_2$ is a common used semiconductor nanomaterial for many applications in environmental fields, including dye-sensitized solar cells [1, 2], water photoelectrolysis [3, 4] and photocatalytic degradation [5, 6] due to its high oxidizing activity of photogenerated holes, low cost, nontoxicity,
physical and chemical stability. Well aligned TiO\textsubscript{2} nanotube arrays (NTAs) were synthesized by Grimes via anodizing Ti sheets in HF solution [7]. The vertically oriented TiO\textsubscript{2} NTAs became a competing substrate in sensors, including gas sensor [8, 9], COD sensor [10, 11] and biosensor [12, 13], due to the special architecture of nanotube arrays.

The commercial biosensor, such as blood glucose meter, is composed of separate electrodes and enzymes, which requires consumption of enzyme in each determination, resulting in high use-cost. Immobilizing the enzymes on the electrode directly can solve this problem. Since Clark and Lyons first proposed the concept of a biosensor where an enzyme is incorporated on electrode surface [14], the immobilized biosensors have attracted great attentions.

Many nanomaterials have been employed as supports for immobilizing enzymes and developing enzyme-based biosensors. The immobilizing amount of enzymes, activity of immobilized enzymes and conductivity of the supports are the key factors for the sensitivity of biosensors. Well-organized TiO\textsubscript{2} NTAs possess good biocompatibility, environmental safety and large surface area. Also, TiO\textsubscript{2} NTAs are easy to coordinate with amine and carboxyl groups on the surface and act as an electron mediator, which facilitates the electron transfer between the redox centers of the enzymes and the electrode surface [15-17]. Thus, TiO\textsubscript{2} NTAs can be used as matrix to immobilize proteins and enzymes for biosensor. Xiao and co-workers reported the fabrication of TiO\textsubscript{2} NTAs by anodization of titanium foil for H\textsubscript{2}O\textsubscript{2} biosensor design [18].

Many efforts have been devoted on the synthesis and modification of TiO\textsubscript{2} TNAs for enhancing the electrochemical performance of TiO\textsubscript{2} NTAs, such as metal nanoparticles modification (including Ag, Au and Pt) [19, 20], semiconductor quantum dots modification (including Cu\textsubscript{2}O and CdS) [21, 22] and carbon nanostructures modification (including carbon nanotubes and graphene) [23, 24], which can provide better electron transportation and show an excellent capability to immobilize enzyme as well. In our previous work, TiO\textsubscript{2} NTAs based biosensors were synthesized and modified with Ag [25], Pt nanoparticles and graphene nanosheets [26]. The modification on TiO\textsubscript{2} nanotubes enhances the charger transfer between the electrolyte and the electrode, hence, enhance the detection performances of the biosensors. These efforts enhance the electrochemical performance of TiO\textsubscript{2} NTAs by external modification. The internal structure of TiO\textsubscript{2} nanotubes is the other key factor for electrochemical performances, including the crystal structure, geometric dimensioning and the surface condition. For example, the different exposed lattice planes of TiO\textsubscript{2} nanocrystals possess different catalytic reaction activity [27].

Great efforts have been dedicated to synthesize TiO\textsubscript{2} nanomaterials with controllable morphology
and porous structure [28]. In particular, mesoporous TiO$_2$ is a kind of attractive semiconductor allowing the reduction of various electron acceptors such as viologen, as well as electron transfer with biological molecules such as flavin coenzymes [29, 30]. Bao and co-workers first developed a novel TiO$_2$ with uniform porous structure via using multi-walled carbon nanotubes as template [31]. Serge Consier et al. for the first time described the functionalization of a mesoporous TiO$_2$ film immobilized with glucose oxidase for the amperometric detection of glucose [32].

In consideration that enzyme molecules contain lots of secondary branched structure, which requires extending room for maintaining its activity [33]. Mesopores on the nanotubes can act as cell to offer the required room. To our knowledge, the introduction of mesopores in TiO$_2$ NTAs, which is suitable for holding enzymes and accelerating charges transfer, has never been reported for biosensor application.

Here, TiO$_2$ NTAs with mesoporous structures were obtained by anodization method combined with chemical etching in HF solution. The mesoporous TiO$_2$ NTAs were immobilized with glucose oxidase (GOx) by physical adsorption and used for glucose determination.

## 2 Experimental

### 2.1 Chemicals and instruments

GOx was purchased from Sigma and used as received without further purification. Ethylene glycol (EG), hydrogen peroxide (H$_2$O$_2$), glucose, phosphate, ammonium fluoride (NH$_4$F), hydrogen fluoride (HF) and other reagents, were analytical reagent grade, and were purchased from Enterprise Group Chemical Reagent Co, LTD. Titanium foils (0.1 mm thickness, 99.6% purity) were purchased from Beijing Cuibolin Non-Ferro Technology Developing Co. LTD and used as received.

A 0.05 M phosphate buffer solution (pH=7) containing Na$_2$HPO$_4$ and NaH$_2$PO$_4$ was used as supporting electrolyte. The enzyme solution was prepared by dissolving GOx in 0.05 M phosphate buffer solution (PBS) to make a 500 U·mL$^{-1}$ solution and was kept at 4°C in the fridge.

Anodization of TiO$_2$ NTAs was performed in a self-made electrolytic cell with a traditional two-electrode system (DH1722A-3). Morphologies of the as-prepared samples were observed with scanning electron microscope (SEM, SV8020) and high resolution transmission electron microscope (HRTEM, JEM-2100F). X-ray diffraction patterns of the samples were recorded at room temperature with 20 angle ranging from 10 to 80° (XRD, D/MAX2500V). The existence valence of TiO$_2$ NTAs was determined by X-ray photoelectron spectrometer (XPS, CALAB250). Cyclic voltammetry and glucose detection were performed by an electrochemical workstation (CHI660D), comprising an
Ag/AgCl (3 M KCl) reference electrode, a Pt/Ti wire auxiliary electrode and a Pt disk working electrode fixed with as-prepared samples.

2.2 Synthetic procedure

Prior to the anodization, Ti foil was rinsed in ethanol and water with ultrasonic vibration for each 5 min. Ti foil was put into a two-electrode electrolytic cell as the working electrode, and a graphite electrode was used as the counter electrode with the distance of 2 cm. Anodic oxidation of Ti foil was performed in an EG solution containing 0.15 M NH₄F and 5% H₂O at a voltage of 60 V for 6 h. Then TiO₂ NTAs supported on Ti substrate were obtained and washed several times to remove the residual solution. The samples were ultrasonic vibrated in EG for 1 min to remove the debris covering on the top surface of TiO₂ NTAs. The dried TiO₂ NTAs were annealed in a muffle furnace at 500°C for 2 h to get anatase TiO₂ NTAs.

The mesoporous structure of TiO₂ NTAs was constructed by chemical etching method in HF solution. The solutions with 0.2:10:89.8, 0.35:10:89.65, 0.5:10:89.5 and 1:10:89 volume ratio of HF, H₂O to EG were prepared respectively. Then 10 mL as-prepared solutions were transferred to teflon-lined stainless steel autoclave containing the anatase TiO₂ NTAs film inside. The autoclaves were then sealed and maintained at 100°C for 5 h to complete the chemical etching process. Then TiO₂ NTAs film was cleaned in deionized water several times and dried at room temperature. The mesoporous TiO₂ NTAs prepared with different HF concentrations of 0.2%, 0.35%, 0.5% and 1% were defined as TiO₂-0.2, TiO₂-0.35, TiO₂-0.5 and TiO₂-1 NTAs, respectively.

The TiO₂ NTAs were fixed on the Pt disk electrode with silver conductive adhesive and a small cap, leaving a circular area of 0.2 cm² as working surface. GOx was immobilized on TiO₂ NTAs by physical adsorption method. In a typical procedure, 10 µL GOx solution with concentration of 500 U·mL⁻¹ were dropped on the electrode. Then the electrode was left in the fridge at 4°C overnight, following with immersion in buffer solution to remove the free GOx before electrochemical detection. The GOx/TiO₂ NTAs electrodes were then achieved and were stored at 4°C in a fridge when not used.

2.3 Electrochemical test and glucose determination

The electrochemical properties of the as-prepared TiO₂ and mesoporous TiO₂ NTAs before and after GOx immobilization were characterized via cyclic voltammetry. The cyclic voltammetry testing was carried out in 0.05 M buffer solution, 10 mM H₂O₂ and 10 mM glucose at a scan rate of 10 mV/s. The amperometry determination of glucose was performed by successively injecting glucose with
different concentrations into 4 mL PBS, under constant stirring (100 rpm) at room temperature.

3 Results and discussion

3.1 Characterization of mesoporous TiO₂ NTAs

Fig.1 shows XRD patterns of TiO₂ NTAs before and after chemical etching. The anodized TiO₂ NTAs are in amorphous structure, which need to be crystalized by annealing method at 500°C for 2 h. The diffraction peaks at 25.3° and 37.8° can be indexed to (101) and (004) lattice planes of anatase TiO₂, indicating that TiO₂ NTAs are in anatase structure. Some small peaks at 35.1°, 40.1° and 52.9° are the diffraction peaks of Ti substrate. The XRD pattern of mesoporous TiO₂-0.5 NTAs is almost the same with that of un-etched TiO₂ TNAs, indicating that the chemical etching process has no influence on the crystal structure of TiO₂ NTAs.
Fig. 2 XPS patterns of as-prepared TiO$_2$ NTAs and mesoporous TiO$_2$-0.5 NTAs, (i) survey patterns, (ii) F 1s electron enlarged patterns.

The commonly used XPS is applied to identify the elemental chemical state in TiO$_2$ NTAs and mesoporous TiO$_2$-0.5 NTAs, as shown in Fig. 2. Fig. 2 (i) shows the survey patterns of the two samples. The sharp peaks of O 1s and Ti 2p appeared at 530.02 eV and 458.45 eV are detected to confirm the major ingredients of the sample. Peak of C 1s at 284.80 eV, existing in the sample, is originated from the testing process of XPS. Peak at 679.58 eV corresponding to F$^-$ ions may originates from the anodization process or chemical etching process.

For further studying the state of F$^-$ and making it clear whether F$^-$ ions physically absorb on the surface or substitute in the crystal lattice of mesoporous TiO$_2$-0.5 NTAs, Fig. 2 (ii) compares scan spectra of F 1s between TiO$_2$ NTAs before and after etching. Two F 1s peaks of TiO$_2$ and mesoporous TiO$_2$-0.5 NTAs are almost the same in intensity and binding energy, which are similar to the physically absorbed F$^-$ on the surface of TiO$_2$ NTAs [34]. The similar state of the F$^-$ ions on TiO$_2$ NTAs before and after etching confirms that F$^-$ ions originate mainly from the anodization process.
Fig. 3 shows SEM morphologies of TiO₂ and mesoporous TiO₂ NTAs prepared in HF solution with different concentrations. Fig.3 (i) is the top view of TiO₂ NTAs, from which the well aligned and closely packed TiO₂ nanotubes with the uniform tube diameter of 120 nm can be observed. It also can be observed in the profile view that the length is approximately 20 μm, as shown in the top right inset. The magnified profile views of TiO₂ and mesoporous TiO₂ NTAs are shown in Fig.3 (ii), (iii), (iv), (v) and (vi), respectively. No obvious mesopores on the tube walls of as-prepared TiO₂, mesoporous TiO₂-0.2 and TiO₂-0.35 NTAs can be observed in Fig.3(ii), (iii) and (iv). In consideration of the closely packed TiO₂ nanotubes, the inner surfaces of the nanotubes are the main sites of chemical etching. Only the mesopores through the tube walls can be observed in the outer surface of TiO₂ nanotubes. So when TiO₂ NTAs were etched in 0.5% HF solution, these mesopores can be observed in the outer walls of the nanotubes, as shown in Fig.3 (v), indicating the higher degree of chemical etching in higher HF concentration. Fig.3 (vi) also shows the morphology of mesoporous TiO₂-1 NTAs, in which large amount of mesopores distribute on the wall of the TiO₂ nanotubes. However, the severe destructing of the mesoporous TiO₂-1 NTAs make the film easily broken during the electrochemical test, and TiO₂-1 NTAs were not used in the further experiments.
Fig. 4 TEM morphologies of as-prepared TiO$_2$ NTAs and mesoporous TiO$_2$-0.5 NTAs, (i) as-prepared TiO$_2$ NTAs, (ii) mesoporous TiO$_2$-0.5 NTAs, (iii) mesoporous TiO$_2$-0.5 NTAs with higher magnification, (iv) HRTEM morphology of mesoporous TiO$_2$-0.5 NTAs

For further studying the inner structure of mesoporous TiO$_2$-0.5 NTAs, HRTEM tests are carried out, and the morphologies are shown in Fig.4. Fig.4 (i) shows the as-prepared TiO$_2$ nanotubes with the diameter of 120 nm, which corresponds to the morphology in Fig.3 (ii). Compared with TiO$_2$ nanotubes in fig.4 (i), there are lots of mesopores distributing in the walls of TiO$_2$ nanotubes after being etched, as shown in Fig.4 (ii). These mesopores are almost rectangle with size of above 10 nm with the sides parallel to the (101) plane of anatase TiO$_2$ as shown in Fig.4 (iii) and (iv). Mesopores are rectangle rather than sphere, which can be attributed to the (101) planes with relative low surface energy to minimize the overall surface energy, which has been confirmed by the fact that natural anantase crystal exposes (101) planes.

There are two kinds of surface species, Ti-OH$_2^+$ and Ti-OH on the surface of TiO$_2$ nanotubes, when anatase TiO$_2$ NTAs are put into the deionized water [35]. And the F$^-$ ions addition converts surface species from Ti-OH$_2^+$ and Ti-OH to Ti-F. The corresponding surface reactions were considered as follows [36]:

\[
\text{Ti-OH$_2^+$ + F}^- \rightarrow \text{Ti-F} + \text{OH}^-
\]

\[
\text{Ti-OH + F}^- \rightarrow \text{Ti-F} + \text{OH}^-
\]
\[
\text{Ti-OH}_2^+ \rightarrow \text{Ti-OH} + \text{H}^+ \quad (1)
\]

\[
\text{Ti-OH} + \text{H}^+ + \text{F}^- \rightarrow \text{Ti-F} + \text{H}_2\text{O} \quad (2)
\]

It is apparent that the adsorption of fluoride on TiO\(_2\) replaces the surface hydroxyl groups to Ti-F species, and the chemical etching of anatase TiO\(_2\) NTAs can be attributed to the production of TiF\(_4\) in the hydrothermal condition.

### 3.2 Electrochemical performances of mesoporous TiO\(_2\) TNAs

![CVs of TiO\(_2\) and mesoporous TiO\(_2\) NTAs](image)

Fig. 5 shows typical cyclic voltammograms (CVs) of TiO\(_2\) and mesoporous TiO\(_2\) NTAs in the electrolyte containing 10 mM K\(_3\)[Fe(CN)\(_6\)] and 0.1 M KCl. A pair of redox peaks can be observed for TiO\(_2\) and mesoporous TiO\(_2\) NTAs at the potentials ranging from 0 to 0.6 V due to the oxidation and reduction of [Fe(CN)\(_6\)]\(^{3-}\) ions.

For a reversible process, Randles-Sevick Equation is applicable. Therefore, the electrochemical active surface areas of TiO\(_2\) NTAs and mesoporous TiO\(_2\) NTAs can be determined by equation (3).

\[
I_p = (2.69 \times 10^5)n^{3/2}AD_0^{1/2}v^{1/2}C_0^* \quad (3)
\]

Where \(I_p\) is the peak current, \(n\) is the number of electrodes, \(A\) is the electrochemical effective surface area of the electrode (in cm\(^2\)), \(C_0^*\) is the concentration (in mol/cm\(^3\)), \(D_0\) is the diffusion coefficient (in…
cm²/s) and ν is the scan rate (in V/s). The peak current (I_p) corresponds to the electrochemical effective surface area (A) of the electrodes.

All mesoporous TiO₂ NTAs exhibit higher peak intensities and larger capacitance than that of as-prepared TiO₂ NTAs, indicating that mesoporous TiO₂ NTAs possess larger electrochemical active surface areas. The peak current increases with the increase of HF concentration ranging from 0.2% to 0.35% and achieves the maximum of 1.1 µA in the HF concentration of 0.35%. Further increase of the concentration to 0.5% will decrease the peak current to 0.43 µA. The change of the peak currents corresponds to the change of the electrochemical active surface area, that is, the mesoporous TiO₂-0.35 NTAs possess the biggest electrochemical active surface area.

![Graph](image)

**Fig.6** EISs of as-prepared TiO₂ NTAs, mesoporous TiO₂-0.2 NTAs, TiO₂-0.35 NTAs and TiO₂-0.5 NTAs in buffer solution

Electrochemical impedance spectrum (EIS) is used to investigate the electrode process and the diffusion kinetics as well as mass parameter. The semicircle part of the Nyquist plot gives the information about capacitive and resistance, and the linear part shows the diffusion effects. The semicircle diameter at higher frequencies corresponds to the charge transfer resistance (R_{ct}), and the linear part at lower frequencies corresponds to the diffusion process.

Fig.6 exhibits EIS plots of different electrodes in the frequency range from 10^2 to 10^5 Hz at potential of 0.1 V. The electrodes exhibit almost straight lines in the lower frequencies which
corresponds to the diffusion process. The $R_{ct}$ of mesoporous TiO$_2$ NTAs is lower than that of as-prepared TiO$_2$ NTAs, and decrease with the increase of HF solution, which means that the mesoporous TiO$_2$ NTAs possess higher electron transfer rate than that of un-etched TiO$_2$ NTAs. The electron transfer rate of mesoporous TiO$_2$ NTAs increases with the concentration ranging from 0.2% to 0.5%, which is different with the trends of the electrochemical active surface area.

In consideration that the quantification of glucose is based on the electrochemical detection of the enzymatically liberated H$_2$O$_2$, the electrochemical response to H$_2$O$_2$ of the electrode is a key factor for the sensor’s sensitivity. High catalytic efficiency to H$_2$O$_2$ is benefit to achieve high sensitivity toward glucose. Here, H$_2$O$_2$ is used as a probe to determine the catalytic property of different TiO$_2$ NTAs electrodes.

![Graph showing CVs of as-prepared TiO$_2$ NTAs (i), mesoporous TiO$_2$-0.2 NTAs (ii), TiO$_2$-0.35 NTAs (iii) and TiO$_2$-0.5 NTAs (iv) in buffer solution in absence and presence of 10 mM H$_2$O$_2$](image)

Fig. 7 shows the CVs of as-prepared TiO$_2$ NTAs (i), mesoporous TiO$_2$-0.2 NTAs (ii), TiO$_2$-0.35 NTAs (iii), and TiO$_2$-0.5 NTAs (iv) in buffer solution in absence and presence of 10 mM H$_2$O$_2$.

When H$_2$O$_2$ is added in buffer solution, additional anodic and cathodic currents can be obtained due...
to the H₂O₂ oxidation and reduction on TiO₂ NTAs. Fig.7 (i) shows the comparison of CVs obtained on TiO₂ TNAs in buffer and in 10 mM H₂O₂ solutions, from which the anodic current increment of 1.787 µA at 0.7 V can be observed. All the mesoporous TiO₂ NTAs possess higher current responses than that of un-etched TiO₂ NTAs, such as 2.46, 3.204 and 3.771 µA for TiO₂-0.2 (ii), TiO₂-0.35 (iii), and TiO₂-0.5 (iv) NTAs respectively. The higher current increments of mesoporous TiO₂ NTAs indicates better catalytic efficiency of H₂O₂ oxidation.

In addition, the maximum current response for H₂O₂ oxidation is achieved by TiO₂-0.5 NTAs, which is consistent with the EIS results in Fig.6, indicating that the best electron transfer ability induces the highest electrocatalytic efficiency of H₂O₂.

3.3 CVs of mesoporous GOx/TiO₂ NTAs

GOx was immobilized on TiO₂ and mesoporous TiO₂ NTAs by physical adsorption to achieve glucose biosensor, and defined as GOx/TiO₂ NTAs. In general, biosensor enzymes are immobilized on the sensors by either cross-linking with glutaraldehyde or being protected with a thin layer of Nafion to prevent the enzymes from losing. Here, the nanotubular structure of TiO₂ NTAs and the highly dispersible mesopores in the tube walls provide a suitable structure for immobilization of GOx. The CVs of TiO₂ and mesoporous TiO₂ NTAs before and after being immobilized with GOx in buffer solution in absence and presence of 10 mM glucose are shown in Fig.8.
Fig. 8 CVs of as-prepared TiO$_2$ NTAs and mesoporous TiO$_2$ NTAs before and after being immobilized with GOx in buffer solution in absence and in presence of 10 mM glucose, TiO$_2$ NTAs (i), TiO$_2$-0.2 NTAs (ii), TiO$_2$-0.35 NTAs (iii), TiO$_2$-0.5 NTAs (iv).

Fig. 8 (i) shows the CVs of as-prepared TiO$_2$ NTAs in different conditions. Curve (a) and (b) compare the CVs of TiO$_2$ NTAs in buffer and in 10 mM glucose. Similarity of the two curves indicates that there is no electrochemical activity of TiO$_2$ NTAs for glucose oxidation in the testing potential region. When GOx is immobilized on TiO$_2$ NTAs, the obvious current response in positive potentials can be observed, as being compared in curve (c) and (d). The current increment at potential of 0.7 V is 0.145 µA. The cathodic currents at low potentials decrease in glucose solution compared with that in buffer, indicating that cathodic current response cannot be used for glucose determination.

Fig. 8 (ii), Fig. 8 (iii) and Fig. 8 (iv) show the CVs of mesoporous TiO$_2$-0.2, TiO$_2$-0.35 and TiO$_2$-0.5 NTAs in different conditions. Similar with TiO$_2$ NTAs, the mesoporous TiO$_2$ NTAs possess no activity of direct electrochemical oxidation of glucose. After being immobilized with GOx, anodic current responses of 0.215, 0.258 and 0.283 µA at 0.7 V can be obtained, which are higher than that of as-prepared TiO$_2$ NTAs. Also, the current response to 10 mM glucose increase with the HF concentration ranging from 0.2% to 0.5%, corresponding to the electrocatalytic efficiencies of H$_2$O$_2$ in Fig. 7.

3.4 Glucose determination

To evaluate the sensitivity of as-prepared biosensors based on TiO$_2$ NTAs and mesoporous TiO$_2$ NTAs, the typical current responses of the obtained biosensors (GOx/TiO$_2$ and mesoporous GOx/TiO$_2$ TNAs) were determined by amperometry. When the background current in buffer solution is steady, the glucose solution with certain concentration is injected into the buffer solution and the
current response is carried out. All processes are under continuous stirring (100 rpm) condition with an applied potential of 0.7 V, and the results are shown in Fig.9.

Fig.9 Amperometric determination of glucose by GOx/TiO$_2$ NTAs, GOx/TiO$_2$-0.2 NTAs, GOx/TiO$_2$-0.35 NTAs, GOx/TiO$_2$-0.5 NTAs electrodes. (i) Current responses with successive injection of glucose; (ii) Calibration curves; (iii) Lineweaver-Burk-type plots for electrochemical determination of apparent Michaelis-Menten constants

From the comparison of current-time curves in Fig.9 (i), GOx/TiO$_2$ NTAs (curve (a)) show considerably weak response for glucose injection at the applied potential of 0.7 V, while all mesoporous GOx/TiO$_2$ NTAs possess higher current response than that of GOx/TiO$_2$ NTAs. The current response to glucose increases with the HF concentration and achieve the maximum in GOx/TiO$_2$-0.5 NTAs. The calibration plots between the current change and glucose concentration are show in Fig.9 (ii), which can give the determination parameters of these biosensors. The slopes obtained by linear fitting of the data show the sensitivities of the biosensors. The sensitivity of TiO$_2$ NTAs is 0.066 µA·mM$^{-1}$·cm$^{-2}$ (calculated with slope of 0.00246 µA·mM$^{-1}$ and working area of 0.037 cm$^2$) with linear range from 0.1 to 13 mM. The sensitivity of GOx/TiO$_2$-0.5 NTAs is 0.954 µA·mM$^{-1}$·cm$^{-2}$ in the linear range from 0.1 to 6 mM, which is twice that of GOx/TiO$_2$-0.35 NTAs, 4.8
times that of GOx/TiO$_2$-0.2 NTAs and 14.3 times that of GOx/TiO$_2$ NTAs.

The effect of mesopores on the sensitivity enhancement can be seen in the kinetics data on the enzyme reaction. The apparent Michalis-Memen constants, $K_m$, for immobilized GOx can be determined electrochemically by using the modified Linewearer-Burk equation.

$$\frac{1}{i_{ss}} = \left(\frac{K_m}{i_{max}}\right)\left(\frac{1}{C}\right) + \frac{1}{i_{max}}$$

(4)

Where $i_{max}$ and $i_{ss}$ are the currents measured for enzymatic product detection under conditions of saturation and steady state for given substrate concentration $C$. A plot of $1/i_{ss}$ vs $1/C$ will give a straight line with the slope equal to $K_m/i_{max}$ and intercept equal to $1/i_{max}$, as shown in Fig.9 (iii). The lower $K_m$ means the better catalytic action of GOx. The $K_m$ values of GOx/TiO$_2$ NTAs is higher than that of GOx/TiO$_2$-0.5 NTAs. The $K_m$ is affected by two factors, mass diffusion limitation and the reaction kinetics of GOx. Generally, the effect of the two factors cannot be separated in the electrochemical method. At high rotation speed ($\omega > 1600$ rpm) the enzyme operates under catalysis control [37] On the electrode immobilized with GOx, assuming that the mesopores do not have effect on the intrinsic catalysis property of GOx, the difference of $K_m$ can be attributed to the mass diffusion limitation of O$_2$ and H$_2$O$_2$ through the mesopores. The higher porosity of TiO$_2$-0.5 NTAs shows a higher mass diffusion rate than that of TiO$_2$ NTAs.

Fig.10 Selectivity test of GOx/ TiO$_2$-0.5 NTAs electrode with additions of 1 mM uric acid, 1 mM ascorbic acid and 1 mM glucose

Selectivity of this biosensor was investigated by testing the amperometric response with injections
of 1 mM uric acid, 1 mM ascorbic acid and 1 mM glucose, in which the previous two components are among the potential interfering electroactive species, as shown in Fig.10. 1 mM uric acid did not give any observable current response. And 1 mM ascorbic acid gave a response of 0.0005 µA, which is 1.39% to the current response of 1 mM glucose (0.036 µA), indicating a good selectivity of the biosensor.

To detect the stability of the GOx/TiO$_2$-0.5 NTAs electrode, CVs of the electrode in 10 mM glucose solution were performed for 20 circles, as being shown in Fig.11. The CVs of the electrode in buffer solution are also listed in this figure for comparison. The current response is 0.283 µA for the first circle and 0.265 µA for the last circle. Only 6.36% response loss indicates the excellent stability of the GOx/TiO$_2$-0.5 NTAs electrode.

Fig.11 CVs of GOx/TiO$_2$-0.5 NTAs in buffer solution and in 10 mM glucose solution for 20 cycles

3.5 Mechanism discussion
XPS results in Fig.3 have confirmed the similarity of the F⁻ ions in TiO₂ NTAs before and after being etched, which indicates that enhancements of the electrochemical performances after chemical etching are induced by the mesoporous structure instead of the absorbed F⁻ ions. The mesoporous structure of TiO₂ NTAs constructed by chemical etching method can enhance the electrochemical performances, hence, enhance the current response to glucose. The excellent sensitivity can be attributed to mesoporous structure of TiO₂ NTAs by the synergetic effects of providing more electrochemical active surface area and higher electron transfer rate. The schematic diagram of glucose detection on mesoporous GOx/TiO₂ NTAs is shown in Fig.12.

The catalytic oxidation of glucose based on the immobilized GOx can be explained as follows:

\[
\text{Glucose} + \text{O}_2 \xrightarrow{\text{GOx}} \text{Gluconic acid} + \text{H}_2\text{O}_2 \quad (5)
\]

In other words, chemical reaction of glucose with GOx is the first step of glucose determination, which produces H₂O₂ in the presence of dissolved oxygen. The enzymatic reaction requires abundant dissolved oxygen and large amount of active enzymes.

In consideration that the scale of mesopores about 10 nm is similar to that of GOx, the extending room is benefit for immobilizing enzymes and maintaining its activity. The active surface available for enzymes immobilizing is enhanced due to the increase of surface area resulting from the mesoporous structure [38]. That is, the mesoporous structure can offer high enzymes loading without serious loss of activity, meeting the requirements of the first step well.

The second step of glucose determination is obtaining the current response of H₂O₂ liberated by enzymatic reaction by the following reaction:

Fig.12 Schematic diagram of glucose detection on mesoporous GOx/TiO₂ NTAs
\[ \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^- \quad \text{(anodic response)} \] (6)

The electrocatalytic oxidation of \( \text{H}_2\text{O}_2 \) takes place on the electrode when applying positive potential, and the anodic current response corresponding to \( \text{H}_2\text{O}_2 \) concentration can be obtained. Hence, high electrochemical activity to \( \text{H}_2\text{O}_2 \) oxidation is benefit for the electrodes achieving high current response to glucose. The CVs in Fig.7 have confirmed that mesoporous TiO\(_2\) NTAs possess higher current response to \( \text{H}_2\text{O}_2 \), meeting the requirement of the second step of glucose detection.

One thing should be noted is the effects of electrochemical active surface area and electron transfer rate on the electrochemical oxidation of \( \text{H}_2\text{O}_2 \). Fig.5 and Fig.6 confirm that mesoporous TiO\(_2\) NTAs possess higher electrochemical active surface area and electron transfer rate than that of un-etched TiO\(_2\) NTAs respectively. However, the highest surface area appears in TiO\(_2\)-0.3 NTAs, and the highest electron transfer rate in TiO\(_2\)-0.5 NTAs. Higher current response to \( \text{H}_2\text{O}_2 \) of TiO\(_2\)-0.5 NTAs than that of TiO\(_2\)-0.3 NTAs (shown in Fig.7) indicates the electron transfer rate plays more important role in the electrochemical process of \( \text{H}_2\text{O}_2 \) oxidation. Also, twice glucose sensitivity of GOx/TiO\(_2\)-0.5 NTAs to GOx/TiO\(_2\)-0.3 NTAs confirms this result.

4 Conclusions

In this work, mesoporous TiO\(_2\) NTAs are successfully synthesized by chemical etching in HF solution and used as basis of biosensor for glucose determination. Effect of HF concentration on the mesoporous structure and electrochemical activity are also discussed. Mesopores with rectangle shape on TiO\(_2\) nanotubes enhance the electrochemical active surface area and electron transfer rate of TiO\(_2\) NTAs. And the electron transfer rate plays more important role than active surface area in sensitivity of the biosensor. Mesoporous TiO\(_2\)-0.5 NTAs immobilized with GOx possess the maximum sensitivity of 0.954 \( \mu \text{A} \cdot \text{mM}^{-1} \cdot \text{cm}^{-2} \) with linear range from 0.1 to 6 mM, which is 14.3 times that of un-etched GOx/TiO\(_2\) NTAs.

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References


Novel mesoporous structures on TiO$_2$ nanotube arrays are achieved for enhancing the electrochemical performances.