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Novel Tungsten Carbide Nanorods: An Intrinsic Peroxidase Mimetic with High Activity and Stability in Aqueous and Organic Solvents

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Abstract

Tungsten carbide nanorods (WC NRs) are demonstrated for the first time to possess intrinsic peroxidase-like activity towards typical peroxidase substrates, such as 3, 3', 5, 5'-tetramethylbenzidine (TMB) and *o*-phenylenediamine (OPD) in the presence of hydrogen peroxide (H_2O_2). The reactions catalyzed by these nanorods follow the Michaelis-Menten kinetics. The excellent catalytic performance of WC NRs could be attributed to their intrinsic catalytic activity to efficiently accelerate the electron-transfer process and facilitate the decomposition of H_2O_2 to generate more numbers of reactive oxygen species (ROS). Based upon the strong peroxidase-like activity of these WC NRs, a colorimetric sensor for H_2O_2 is designed, which provides good response towards H_2O_2 concentration over a range of 2×10^{-7} to 8×10^{-5} M with a detection limit of 60 nM. Moreover, the peroxidase-like activities of WC NRs with TMB as the substrate are investigated in both protic and aprotic organic media, showing different colorimetric reactions from that performed in aqueous solutions. In comparison with the natural horse radish peroxidase, WC NR exhibits excellent robustness of catalytic activity and considerable reusability, thus making it a promising mimic of peroxidase catalysts.

Keywords: Colorimetric sensor; Nanomaterials-based artificial enzyme; H_2O_2 detection; Peroxidase-like activity; Tungsten carbide nanorods.

1. Introduction

Peroxidase activity is essential to many biochemical assays for their application in biomedicine or environmental monitoring. Currently, most of these assays use natural enzyme peroxidases, among which horse radish peroxidase (HRP) is popularly adopted. In spite of their high activity, the wide applications of enzyme peroxidases have been restricted by their limited natural sources, high production costs and low stability (Wagner et al. 1995). Artificial enzyme mimic therefore attracts great attention in the past decades because of its applicability in a wide range of pH, temperature and chemical environment. The organometallic complexes including hemin, hematin and porphyrin have been utilized as peroxidase mimetic (Johnstone et al. 1997; Wang et al. 2007) in the earlier research. Recently, nanomaterials that possess peroxidase activity have received a widespread interest due to their reliable reactivity, easier fabrication and storage than organic enzyme mimics. Numerous nanomaterials such as magnetic nanoparticles and nanocomposites (Dai et al. 2009; Gao et al. 2007; Gao et al. 2013; Lee et al. 2013; Park et al. 2011), metals and alloys (Bernsmann et al. 2011; Jv et al. 2010; Sun et al. 2013; Zhang et al. 2013; Zhou et al. 2013), metal oxides and sulfides (Asati et al. 2009; Chen et al. 2012; He et al. 2012; Hong et al. 2013), carbon materials (Li et al. 2013; Shi et al. 2011; Song et al. 2010; Zheng et al. 2013) and others (Lin et al. 2013; Wang et al. 2013) are reported for their intrinsic peroxidase-like activities in bioassays. However, some concerns regarding to their robustness, large-scale feasibility and expensive cost still remain. Moreover, nanomaterial-based peroxidase mimics are expected to be more convenient and versatile than natural enzymes in non-aqueous applications. For example, when HRP is used to catalyze the polymerization of phenols or aniline, it requires a complicated and time-consuming process, such as chemical modification or supported immobilization of HRP (Takahashi et al. 2001), in order to retain its enzyme activity

in organic media. However, studies on the catalytic properties of peroxidase nanomimics in non-aqueous media are still scarce (Andre et al. 2011). Thus, there is a big motivation to explore novel nanomaterials-based enzyme mimics to be used under unusual or challenging conditions.

Tungsten carbide (WC) as catalyst for electron-transfer reactions has been extensively investigated in various applications, such as, hydrogenolysis and isomerization reactions, fuel cells, hydrogen evolution and oxygen reduction (Palanker et al. 1976; Rosenbaum et al. 2006), because it exhibits catalytic properties similar to those of noble metals (Levy and Boudart 1973). Based on the distinctive advantages, for example low cost, excellent corrosion resistance, high melting point and good electrical conductivity, WC materials have the potential for a large range of applications in biology (Rosenbaum et al. 2006). To our knowledge, the investigation of WC materials as artificial enzyme mimics for bioassay has not been reported. Herein, we demonstrate for the first time that the intrinsic catalytic activity of WC nanorods (WC NRs) towards typical peroxidase substrate, such as 3, 3', 5, 5'-tetramethylbenzidine (TMB) and *o*-phenylenediamine (OPD) in the presence of hydrogen peroxide (H_2O_2). A colorimetric method is developed for simple, fast and sensitive detection of H_2O_2 using WC NRs. The kinetic parameters of TMB oxidation catalyzed by WC NRs are obtained and compared with that of natural HRP. The possible mechanism for the intrinsic peroxidase-like properties of these WC NRs is proposed. Moreover, the catalytic behavior of WC NRs in various organic media is also studied. Possessing excellent catalytic activity, durable stability and good reusability, WC NRs would be a promising peroxidase mimic.

2. Material and methods

2.1. Materials

Sodium tungstate dihydrate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$), Ammonium sulfate (NH_4SO_4), glucose, TMB, OPD, Rhodamine B (RhB), Orange II, natural HRP (type II, 150-250 units/mg), H_2O_2 , Tungsten (IV) carbide and Sodium acetate anhydrous (NaAC) were purchased from Sigma-Aldrich (Singapore). Hydrochloric acid (HCl), acetic acid (HAC) and organic solvents (methanol, isopropanol and acetonitrile) were purchased from Merck (Singapore). Unless otherwise stated, all of the chemicals were used without further purification. Millipore Milli-Q water ($18 \text{ M}\Omega \text{ cm}^{-1}$) was used in all experiments.

2.2. Synthesis of WC NRs

The unique nanorod-structured WC materials are prepared as described elsewhere (Yan et al. 2012). Briefly, $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ (0.6579 g) and $(\text{NH}_4)_2\text{SO}_4$ (0.5286 g) were dissolved in 15 mL deionized water, then HCl aqueous solution (3 M) was added under stirring until pH value to 2.0. The solution was maintained in Teflon-lined stainless steel autoclave at 180°C for 8h. Then the precipitate was filtered, washed several times with water and ethanol and dried at 60°C to obtain WO_3 nanorods. The as-prepared WO_3 nanorods and glucose (molar ratio W/C=0.078) were dissolved in 15mL deionized water and stirred for 20min before hydrothermal treatment at 180°C for 8 h. Finally, the as-prepared precursors of carbon-coated WO_3 nanorods were calcined at 900°C under a flow of H_2/Ar ($V_{\text{H}_2}/V_{\text{Ar}}=1:3$, 300 mL min^{-1}) for 3h to form nanorod-structured WC materials. The as-prepared WC NRs were analyzed by field emission scanning electron microscopy (FESEM) and high-resolution transmission electron microscopy (HRTEM).

2.3. Peroxidase-like activities of WC NRs

The measurements were conducted using a Shimadzu UV-2450 spectrophotometer by recording the adsorption spectra at 654 nm for 0.6 mM TMB or at 450 nm for 0.4 mM OPD in acetate buffer (500 μL , 50 mM, pH 4.0) containing WC NRs 0.02 mg mL^{-1} and H_2O_2 (400 mM,

30% (v/v)). Color reactions were observed immediately after the substrates were added. The photographs and UV absorbance were recorded after the reaction for 20 min to reach the stable state. The control experiments were performed in the absence of substrates or WC NRs, or carried out by using commercially available WC particles. The catalytic oxidation of TMB by WC NRs in the presence of H_2O_2 was also determined under the conditions described before, except that instead of buffer, different organic solvents (acetonitrile, isopropanol and methanol) were selected to prepare all the solutions.

2.4. Colorimetric detection of H_2O_2 using WC NRs

The assay was started by the addition of different concentrations of H_2O_2 into 500 μL NaAC buffer solutions (50 mM, pH 4.0) in the presence of 0.02 mg mL^{-1} WC NRs and 0.6 mM TMB. After the colorimetric reactions were completed, the WC NRs were removed from the reaction solution by centrifugation and the supernatant solution was used for the analysis at 654 nm. The final concentrations of H_2O_2 in the system varied from 0.1 μM to 1 mM.

2.5. Kinetics measurements

Kinetics measurements were carried out at room temperature using 0.02 mg mL^{-1} WC NRs in NaAC buffer solutions (500 mL, 50 mM, pH 4.0) by varying the concentration of TMB (0-0.8 mM) at a fixed concentration of H_2O_2 or vice versa by varying the concentration of H_2O_2 (0-600 mM). All the reactions were monitored in time-scan mode at 654 nm and the apparent kinetics parameters were determined by nonlinear fitting of the absorbance data to the Michaelie-Menten equation, $v = V_{\text{max}} \times [\text{S}] / (K_{\text{m}} + [\text{S}])$, where v is the initial reaction rate, V_{max} is the maximal reaction rate, $[\text{S}]$ is the substrate concentration and K_{m} is the Michaelis-Menten constant (Marquez and Dunford 1997).

2.6. Robustness of peroxidase-like activity of WC NRs

WC NRs were incubated in buffers at various pH ranging from 2-10 for 4h and at various temperatures ranging from 20 to 100 °C for 2h, respectively. Then their catalytic activities were measured under standard experimental condition. For the durability test, WC NRs were incubated in acetate buffer (500 μ L, 50 mM, pH 4.0) at room temperature for 24h, and then their activities were measured under standard condition and compared with that of the freshly-prepared sample. The control experiments of pH value, temperature and time effects on the activity of HRP were performed under the same assay conditions as that for WC NRs, but using 10 ng mL⁻¹ HRP, 0.3 mM TMB and 1 mM H₂O₂.

2.7. WC NRs for peroxide-sensitive electrochemical assay

The peroxide-sensitive electrochemical assay using WC NRs was carried out using an Autolab Potentiostat (Eco Chemie, Netherland) in a three-electrode system in NaAC-HAC buffer (pH 4.0). An Ag/AgCl-saturated KCl electrode and a platinum wire served as the reference electrode and counter electrode, respectively. The working electrode was WC NRs modified glass carbon electrode, which was prepared as below. Briefly, a mixture containing the catalyst (2.0 mg), ethanol (2.5mL), and Nafion solution (0.5 mL, 0.05 w%) was ultrasonicated for 15 min to obtain a well-dispersed ink. Then, a certain amount of such dispersion was transferred onto the surface of the electrode and a catalyst thin film containing WN NRs was obtained after the solvent slowly evaporated. The amperometric currents were recorded at the potential of -0.4 V by successive additions of 33 μ M H₂O₂ in time intervals of 150 s.

3. Results and discussion

3.1. Characterization of WC NRs

WC NRs are synthesized by a template-free pseudomorphic transformation of chemically synthesized WO_3 nanorod through a high-temperature method. As shown in Fig. 1A, the as-prepared WC nanocrystals are generally rod-like in shape with an average diameter of 30-50 nm and **length** of 150-200 nm. The individual nanorod is highly crystalline with lattice fringes of 0.28 nm (Fig. 1B), corresponding to (001) lattice plane of hexagonal WC. **In contrast, the commercial WC is composed of crystals with few-micron size, showing an agglomerated morphology (Fig. 1C).**

3.2. Peroxidase-like activity of WC NRs

In the presence of H_2O_2 , WC NRs can quickly catalyze the oxidation of typical peroxidase substrates, TMB and OPD, producing a blue color for TMB and a yellow color for OPD, respectively (Fig. 2A). **Even though the slow self-decomposition of H_2O_2 could cause the imperceptible oxidation of organic substrates, the addition of the as-prepared WC NRs effectively speeds up the reactions, dramatically increasing the sensitivity of the colorimetric reaction (Fig. S1 in the Supporting Information).** It is speculated that WC NRs as heterogeneous catalysts accelerate the electron-transfer process and facilitate the decomposition of H_2O_2 to generate more numbers of reactive oxygen species (ROS), such as hydroxyl radical ($\cdot\text{OH}$). The schematic illustration of **the** colorimetric reaction is described in Fig. 2B and the possible mechanism will be discussed in detail later. With regard to the TMB oxidation catalyzed by WC NRs, the maximal absorbance is present at 654 nm (Fig. 2C), indicating the formation of TMB cation radicals by one-electron oxidation (Josephy et al. 1982). Neither H_2O_2 nor WC NRs alone can efficiently oxidize TMB (Fig. 2C), suggesting that the interaction between WC NRs and H_2O_2 /TMB is important for the catalytic reaction. For comparison purpose, the catalytic activity of commercial WC was also tested (as shown in Fig. 2C and 2D). The results suggest that the

catalytic activities of WC NRs are much higher than that of commercial WC materials (inset of Fig. 2C). The time-dependent absorbance changes further indicate that the initial oxidation rate of TMB catalyzed by WC NRs is 4 times higher than that catalyzed by commercial WC (Fig. 2D). Similar results are observed for the case of OPD as the substrate (Fig. S2 in the Supporting Information). These results highlight the importance of the nanostructure to the catalytic properties. Thus, the superior catalytic activity of WC NRs would be attributed to the smaller size and well-defined nanorod structure.

3.3 Optimization of experimental conditions

The catalytic activity of WC NRs is dependent on pH, temperature, substrate concentration and catalyst concentration. Like HRP, the catalytic activity of WC NRs in acidic solution is much higher than that in neutral or basic solutions (Fig. 3A), resulting from the acid-promoted colorimetric reaction of TMB (Josephy et al. 1982). Unlike HRP, WC NRs still retain excellent peroxidase activity at high temperature (Fig. 3B). In addition, WC NRs exhibit their peroxidase activity even in the presence of high H_2O_2 concentration (Fig. 3C). It is well known that the activity of HRP is restricted to a narrow range of pH, temperature and chemical environment. In contrast, the as-prepared WC NRs present a stable activity in these conditions that would probably make natural HRP denatured. The experiments were further conducted to obtain the optimal conditions for the maximum catalytic activity of the WC NRs: $20\ \mu\text{g mL}^{-1}$ WC NRs in pH 4.0 buffers containing $400\ \text{mM H}_2\text{O}_2$ and $600\ \mu\text{M TMB}$ at $30\ ^\circ\text{C}$ (Fig. 3D).

3.4 Robustness and reusability of WC NRs

WC NRs possess superior catalytic activity and stability than HRP after the incubation in a wide range of pH (2 to 10) and temperature (4 to $100\ ^\circ\text{C}$) (Fig. 3E and 3F). After such incubation, the activity of HRP decreases dramatically due to the possible denature caused by external

environment changes. Benefiting from their good corrosive resistance (Nikolova et al. 1984), WC NRs is more stable than other peroxidase nanomimics, such as metal oxide/sulfide, which tend to suffer from “metal ion-leaching” at low pH solution. Moreover, more than 90% of catalytic activity of the WC NRs can be retained after the incubation in acid buffers at 100 °C for 2h, suggesting a reliable thermal-tolerance compared with HRP and other reported peroxidase nano-mimics (Table S1 in the Supporting Information). Also, a negligible change on colorimetric results is observed for WC NPs before and after the incubation in pH 4.0 buffer up to 24 h, whereas the activity of HRP almost disappears (Fig. 3G). In terms of the reutilization of WC NRs, the results show that the catalytic activity of WC NRs undergoes a slight decrease after each cycle, yet it still retains 75% after 5 catalytic cycles (Fig. 3H). These results provide strong evidence that WC NRs possess excellent robustness, stable activity and good reusability.

3.5 Kinetics parameters and H_2O_2 detection

To verify the peroxidase-like activity of WC NRs, the steady-state kinetic assays are carried out. The time-dependent UV absorption spectra indicate that the catalytic oxidation of TMB by WC NRs follow the Michaelis-Menten behavior (Fig. S3 in the Supporting Information). In order to get a better insight into the substrate dependency, various UV absorption spectra are obtained by varying one substrate concentration and fixed the other. The slope of the plot is calculated as the initial reaction rate. By plotting the initial reaction rate against concentration, the typical Michaelis-Menten curves for TMB and H_2O_2 are obtained in Fig. 4A and 4B. The kinetic parameters are obtained from Line-weaver-Burk plots (Lineweaver and Burk 1934) and compared with that of natural HRP (Table 1). The value of K_m indicates the affinity strength between the enzyme and the substrates (Marquez and Dunford 1997). WC NRs has smaller K_m value for TMB than that of HRP, indicating a higher affinity of TMB to WC NRs than to HRP.

With H_2O_2 as the substrate, the K_m value of WC NRs is significantly larger than that of HRP. It is consistent with the obtained results that a higher concentration of H_2O_2 is required for the maximal catalytic activity of WC NRs. The double reciprocal plots (Fig. 4C and 4D) are further obtained from the measurements of the WC NRs activity over a range of TMB and H_2O_2 concentrations, which indicates a characteristic Ping-Pong mechanism, as observed for HRP (Rodriguez-Lopez et al. 2001).

Based on the intrinsic peroxidase-like activity of WC NRs, a colorimetric method for H_2O_2 detection is successfully developed. The change of the maximal absorbance of TMB oxidation can be used for H_2O_2 detection. Under the optimum experimental conditions, the absorbance at 654 nm shows a linear increase with the H_2O_2 concentration from 2×10^{-7} to 8×10^{-5} M with a limit of detection (LOD) 60 nM, as shown in Fig. 5A. This provides a simple, low-cost and convenient colorimetric assay for H_2O_2 with high sensitivity (Table S2 in the Supporting Information). In addition, the amperometric responses of a peroxide sensor are measured at a glassy carbon surface modified by WC NRs. A fast, sensitive and well-defined amperometric signal can be obtained by using the WC NRs-based peroxide sensor (Fig. 5B).

3.6 Peroxidase-like activity of WC NRs in organic solvents

Non-aqueous enzymology has provided an exciting tool for synthetic reaction (Dordick 1991). Thus, it is also attractive to study the peroxidase-like activities of WC NRs mimics in organic solvents (acetonitrile, isopropanol and methanol). None of the organic solvents leads to specific reaction between TMB and H_2O_2 in the absence of WC NRs, which further confirms that the catalytic activity is from WC NRs. More interesting observation is that in all the organic solvents investigated, the catalytic oxidation of TMB by WC NRs directly yield light yellow products with the maximal absorbance at 450 nm (Fig. 6A), which is different from the reaction

performed in buffer solution. Moreover, the initial oxidation rate of TMB catalyzed by WC NRs shows 2-fold increase in acetonitrile than in protic organic solvent such as methanol and isopropanol (Fig. 6B). Similar results are also obtained for the catalytic oxidation of OPD in the same organic solvents (Fig. S4 in the Supporting Information). This may be explained by the facilitated decomposition of H_2O_2 and the promoted stability of transition-metal complexes with reactive oxygen species in aprotic media (Todres 1985). As shown in Fig. 6C, it is speculated that, unlike in aqueous solution, it is difficult to retain the equilibrium between the free cation radical from one-electron oxidation of TMB and the charge transfer complex in the aprotic environment. Then, organic solvents may play an important role as an initiator to accelerate the direct two- electron catalytic oxidation of TMB, forming yellow diimine products (Joseph et al. 1982).

3.7. Preliminary investigation for mechanism of WC NRs as peroxidase mimic

It has been reported that the generation of short-lived $\cdot OH$ radicals could be enhanced by WC particles in phosphoric buffer through the electron spin resonance (ESR) studies (Stefaniak et al. 2010). To assess this results, the cost-effective, simple spectrophotometric and fluorescence methods are used to evidence the $\cdot OH$ production. Since RhB and Orange II can be oxidized by $\cdot OH$ to yield photoinactive product (Yu et al. 2008), in the presece of H_2O_2 , the fluorescence quenching of Rhodamine B (RhB) and a decreased absorbance of Orange II under the catalysis of WC NRs are clearly observed (Fig. S5 and S6 in the Supporting Information), which are consistent with the $\cdot OH$ production.

Unlike the redox-based mechanism of Fenton-type reaction for the previously reported peroxidase mimics composed of metal ion (Prousek 2007), the promoted generation of radical by WC NRs could be explained by the surface catalysis mechanism, which depends upon both

particle surface area and surface chemistry (Watanabe et al. 2009). It has demonstrated that the radical generation takes place on some sites of the WC surface activated by atmospheric oxygen (Stefaniak et al. 2010). Due to the large electronegativity of WC (Nwosu 2012), the generated $\cdot$ OH could be stabilized on the WC NRs surface via partial electron exchange interaction (Harriman et al. 1988). Meanwhile, the protonated TMB molecules are easily absorbed on the WC NRs surface and donate partial long-pair electron transferring from its amino groups to the WC NRs, leading to an increase in electron density and mobility in WC NRs (Zhang et al. 2003). Thus, WC NRs could catalytically decompose H_2O_2 to generate $\cdot$ OH, which causes a quick oxidation of organic substrates by a nucleophilic attack and leads to an intense color reaction.

4. Conclusion

In summary, it is found that WC NRs possess an intrinsic peroxidase-like activity in both aqueous and organic media. High affinity towards peroxidase substrates and a typical Michaelis-Menten kinetics are observed. Based on the peroxidase-like properties of these WC NRs, a novel method for H_2O_2 detection is proposed and it provides a simple, low-cost and convenient colorimetric assay for H_2O_2 with high sensitivity. Compared to natural enzyme HRP, WC NRs exhibit superior catalytic activity and good reutilization. With these advantages, WC NRs are promising as a peroxidase mimic for wide applications in biocatalysis, biosensors, and environmental monitoring.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at

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Figures and Captions

Table 1. Michaelis constant (K_m) and maximal reaction rate (V_{max}) of WC NRs and HRP.

Catalyst	Substrate	K_m (mM)	V_{max} (10^{-8} M s $^{-1}$)
WC NRs	TMB	0.274	6.81
WC NRs	H ₂ O ₂	119.6	7.16
HRP ^(Gao et al. 2007)	TMB	0.434	10.00
HRP ^(Gao et al. 2007)	H ₂ O ₂	3.7	8.71

Fig. 1. (A) FESEM images and (B) TEM image of as-prepared WC NRs. Inset of image (B): HRTEM image of an individual nanorod with the lattice fringes of WC (001) plane. (C) SEM image of commercial WC materials.

Fig. 2. The intrinsic peroxidase-like activity of WC NRs: (A) Photographs of oxidation reaction of TMB and OPD; (B) Schematic illustration; (C) UV-vis absorption spectra and (D) Time-dependent absorbance change at 654 nm in the different reaction systems: a) WC NRs + TMB + H_2O_2 ; b) commercial WC + TMB + H_2O_2 ; c) TMB + H_2O_2 ; d) WC NRs + TMB.

Fig. 3. (A) pH, (B) temperature, (C) H₂O₂ concentration and (D) WC NRs concentration effect on the peroxidase-like activity of WC NRs. Experiments were carried out using 20 μg mL⁻¹ WC NRs or 10 ng mL⁻¹ HRP in 0.5 mL in pH 4.0 buffer with 0.6 mM TMB as substrate, except the parameter investigated. The H₂O₂ concentration was 400 mM for WC NRs and 1mM for HRP. (E) pH-dependent stability of WC NRs and HRP after incubation at pH 2 – 10 buffers for 4 h at room temperature; (F) Thermal-dependent stability of WC NRs and HRP after incubation at 4 – 100 °C for 2h in pH 4.0 buffers; (G) Time-dependent stability of WC NRs and HRP before and after incubation for 24 h in pH 4.0 buffer at room temperature, (Inset of G) photographs of color evaluation: (a, b) WC NRs, (c, d) HRP. (H) Reutilization of WC NRs for TMB oxidation catalyzed by WC NRs. The maximum point in each curve is set as 100%.

Fig. 4. Steady-state kinetic assay and catalytic mechanism of WC NRs: (A) The concentration of H_2O_2 is 0.4 M and the TMB concentration is varied; (B) The concentration of TMB is 0.6 M and the H_2O_2 is varied; (C, D) Double reciprocal plots of activity of WC NRs with the concentration of one substrate (TMB or H_2O_2) fixed and the other varied. The error bars represent the standard error derived from three measurements. The relative standard deviations (RSD) for (A) and (B) are 0.979 and 0.983, respectively. And RSD for fitting curves (from top to bottom) in (C) and (D) are 0.989, 0.978, 0.967; and 0.979, 0.991, 0.987, respectively.

Fig. 5. (A) Linear calibration plot between the absorbance at 654 nm and the H_2O_2 concentration.

The error bars represent the standard deviation of three measurements. (B) Amperometric

response of (a) bare GCE and (b) WC NRs modified glass carbon electrode in NaAC-HAC

buffer (pH 4.0) at applied potential of -0.4 V by successive additions of $33 \mu M$ H_2O_2 in the time

intervals of 150 s.

Fig. 6. TMB oxidation catalyzed by WC NRs in organic solvents: (A) UV-vis adsorption spectra and (inset of A) the color photographs; (B) Time-dependent absorbance change at 450 nm in different solvents: (a) acetonitrile, (b) isopropanol and (c) methanol. (C) Scheme of TMB oxidation mechanism in aqueous and organic solvents.

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Tungsten carbide nanorods have been demonstrated for the first time to possess intrinsic peroxidase-like activity to catalytically oxidize the peroxidase substrates in the presence of H_2O_2 in both aqueous and organic media. The colorimetric reactions in organic media are different with that performed in aqueous solutions. Based on the peroxidase-like properties of these WC NRs, a colorimetric sensor for H_2O_2 is designed. With high activity, remarkable stability and considerable reusability, tungsten carbide nanorods would be a promising peroxidase mimic.

ToC figure