A V_2O_3-ordered mesoporous carbon composite with novel peroxidase-like activity towards the glucose colorimetric assay

Lei Han, Lingxing Zeng, Mingdeng Wei, Chang Ming Li and Aihua Liu

It is of great scientific and practical significance to explore inorganic mimetic enzymes to replace natural enzymes due to their instability and high cost. Herein we present an interesting discovery that a V_2O_3-ordered mesoporous carbon composite (V_2O_3-OMC) has a novel peroxidase-like activity towards fast redox reaction of typical peroxidase substrates H_2O_2 and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS). Due to the small size effect and large surface area of V_2O_3 nanoparticles supported by OMC, V_2O_3-OMC exhibited excellent catalytic performance with a k_cat of 1.28 × 10^4 s^{-1}, K_M (ABTS) of 0.067 mM and K_M (H_2O_2) of 0.16 mM, and a significantly higher catalytic efficiency (k_cat/ K_M) towards the oxidation of ABTS in comparison with the natural peroxidases. Furthermore, the Ping-pong BiBi mechanism was proposed to explain the catalytic reaction by V_2O_3-OMC. Based on this highly active biomimetic peroxidase and the colorimetric detection of H_2O_2, a facile analytical method was developed to detect glucose by using V_2O_3-OMC and glucose oxidase, which had a wide linear range (0.01–4 mM glucose), good selectivity and reliability for successful detection of various real samples. Thus, the novel V_2O_3-OMC peroxidase mimic holds great promise for broad potential applications.

Introduction

Enzymes are of great scientific and practical significance in the fields of clinical diagnosis, chemical industry, environmental protection and food production, and have remarkable substrate specificity and high efficiency due to their biological catalytic mechanism. However, most natural enzymes exist as proteins (except for some that are RNAs), which have some disadvantages such as high expense due to their complicated procedure in preparation and purification, easy denaturation under harsh conditions and easy digestion by proteases. Therefore, it is highly desirable to search for enzyme-like mimetics (also called mimetic enzyme) with easy preparation, good cost-effectiveness, high activity and excellent stability to overcome the drawbacks of natural enzymes. To challenge these issues, inorganic nanomaterials such as carbons, metals, and metal-oxides, have widely been used as mimetic enzymes.

So far, many enzymes have corresponding mimetics, such as glycosidase, phosphatase, epoxidase, protease, nuclease, oxidase, peroxidase and superoxide dismutase. Peroxidase is one of the important enzymes and has been widely applied to chemical analysis, medical diagnosis and wastewater treatment. This kind of enzyme generally catalyzes the oxidation of organic electron donors by using hydrogen peroxide (H_2O_2) as the oxidizing agent. The electron donors usually are aromatic compounds and their oxidation can reduce their toxicity or provide a color change, which is detectable by colorimetric methods. Therefore, peroxidases, especially horseradish peroxidase (HRP) and soybean peroxidase (SRP), have been commonly used as the indicator enzyme in analytical techniques such as detection kits for H_2O_2 or glucose, enzyme-linked immunosorbent assay and immunodetection of antigens.

Due to the small size effect, large surface area and high catalytic activity, many nano-structured metal-oxides have been widely used as peroxidase mimetics and subsequently applied in bioanalysis, such as Fe_3O_4 magnetic nano-
particles, bovine serum albumin (BSA)-templated MnO2 nanoparticles, V2O5 nanowires, and C3O2 nanoparticles. Vanadium is a transition metal element and has been found in some organisms such as algae and fungi. It is well known that vanadium is directly relevant to the active center or co-factor of some enzymes, such as vanadium haloperoxidase, vanadium bromoperoxidase and vanadium nitrogenase. Vanadium oxides, mainly including V2O5, VO2 and V2O3, have been extensively studied for their electro-optic properties, field emission, and their use in lithium batteries, catalysts, electrochemical ethanol sensors, photocatalysis, thermochromic smart windows and surface-enhanced Raman scattering. However, there are few reports about the catalytic activity of V2O3 and its composites. Herein, an interesting discovery was made that the V2O3-ordered mesoporous carbon composite (V2O3-OMC) demonstrated excellent peroxidase-like activity, for which a Ping-pong BiBi mechanism was proposed. Based on the colorimetric detection of H2O2, a facile glucose assay was successfully developed by using V2O3-OMC and glucose oxidase (GOx, EC 1.1.3.4).

Experimental section

Chemicals and materials
Hydrogen peroxide (H2O2, 30%) and glucose were purchased from Sinopharm Chemical Reagent Co. 2,2′-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) and GOx were purchased from Sigma-Aldrich. 3,3′,5,5′-Tetramethylbenzidine (TMB) was purchased from TCI (Shanghai) Development Co., Ltd. All other reagents were of the analytical grade and all aqueous solutions were prepared with Milli-Q water (18.2 MΩ cm).

Synthesis and physical characterization of V2O3-OMC
V2O3-OMC was synthesized based on our previous report. In brief, NH4VO3 (30 mg mL−1) and OMC (10 mg mL−1) were sequentially added into concentrated nitric acid (69 wt%) under ultrasonication. After 1 h stirring at 75 °C, the mixture was dried, ground and calcined under an Ar atmosphere at 600 °C for 4 h. The X-ray diffraction (XRD) patterns were recorded on a PANalytical X′Pert spectrometer. Transmission electron microscopy (TEM) images were taken on a FEI F20 S-TWIN instrument. Thermogravimetry analysis (TGA) was performed using a CHNS/O analyzer (PE 2400II) under an air atmosphere and the samples were heated from 50 to 700 °C at a rate of 5 °C min−1. The electron paramagnetic resonance (EPR) measurements were performed using a Bruker A300 EPR spectrometer operated in the X-band (9.842 GHz).

Standard assay for the peroxidase-like activity of V2O3-OMC
To investigate the peroxidase-like activity of the V2O3-OMC, H2O2 and ABTS were chosen as the substrates, where ABTS could be oxidized by H2O2 to a colored product (ABTS+) in the catalysis of the peroxidase mimetics (H2O2 + ABTS → H2O + ABTS+). In a typical experiment, the reaction system (200 μL) contained V2O3-OMC (1 μg), H2O2 (1 mM) and ABTS (0.6 mM) in acetate buffer (100 mM, pH 4.0) at 40 °C, and H2O2 was finally added to start the reaction. The absorbance of ABTS+ at 414 nm (ε144 nm = 36 mM−1 cm−1) was continuously measured for 5 min using a microplate reader (Hitachi) and the initial rates were calculated from the linear portions of the reaction curves.

Peroxidase-like activity characterization of V2O3-OMC
For dependency of the ABTS oxidation rate on the amount of V2O3-OMC, the catalytic activity of the V2O3-OMC was measured by the above assay, except that the amount of V2O3-OMC was varied (0-2 μg) and the absorbance at 414 nm was continuously measured for 5 min. The influences of pH or temperature on the catalytic activity of the Au@Ag NRs were measured by the above assay, except that the pH (1.0–12.0) or temperature (15–60 °C) of the reaction system was changed, respectively. To research the pH- or temperature-stability of V2O3-OMC, V2O3-OMC was incubated in various pH buffers (100 mM, pH 1.0–12.0) at 40 °C for 2 h or in acetate buffer (100 mM, pH 4.0) at different temperatures from 4 to 80 °C for 2 h, separately. After that, the solutions were cooled on ice and then the remaining relative activities were determined by the above assay. Throughout these experiments, the pH values were controlled in the 1.0–12.0 range by using different buffers (100 mM): glycine–HCl buffer (pH 1.0–2.0), acetate buffer (pH 3.0–5.0), phosphate buffer (pH 6.0–8.0), Tris–HCl buffer (pH 9.0–10.0) and NaHCO3–NaOH buffer (pH 11.0–12.0). The maximum activity in each group of experiments was defined as 100% relative activity.

The steady-state kinetics study
For kinetics parameters, the catalytic activity of the V2O3-OMC was measured by the above assay, except for the fixed concentration of ABTS (0.6 mM) and various concentrations of H2O2 (0.01–10 mM) as well as the fixed concentration of H2O2 (10 mM) and various concentrations of ABTS (0.001–0.6 mM). The values of Km and Vmax were calculated by fitting the data to the Michaelis–Menten equation: $v = \frac{V_{\text{max}}}{K_m + [S]}$, where $v$ is the reaction velocity, $V_{\text{max}}$ is the maximal reaction velocity, [S] is the concentration of the substrate and $K_m$ is the Michaelis constant. The turnover frequency ($k_{\text{cat}}$, also called catalytic constant) is calculated using the formula: $k_{\text{cat}} = \frac{V_{\text{max}}}{[V_2O_3-OMC]}$. To study the reaction mechanism, the catalytic activity of the V2O3-OMC was measured by the above assay, except that the concentrations of H2O2 (0.5, 1 and 2 mM) and ABTS (0.03, 0.06 and 0.12 mM) were varied one at a time. The kinetic data were adjusted to the Lineweaver–Burk double reciprocal plot.

Colorimetric detection of H2O2
To detect the concentrations of H2O2, the V2O3-OMC catalytic reactions were carried out using a fixed concentration of ABTS (0.6 mM) and varying H2O2 concentrations (0.01–0.25 mM) as the substrate in acetate buffer (100 mM, pH 4.0) at 40 °C, and then the absorbance at 414 nm were measured after 5 min.
Colorimetric detection of glucose

For the standard glucose assay, GOx-catalytic oxidation of glucose (glucose + O₂ → H₂O₂ + gluconic acid) was coupled with the colorimetric detection of H₂O₂. Briefly, glucose with varying final concentrations (0.05–4 mM) was added into the phosphate buffered saline (PBS, pH 7.4) containing GOx (2 mg mL⁻¹) and the mixture solution (100 μL) was incubated at 37 °C for 30 min. After 30 min, the reaction was stopped by adding 75 μL of acetic buffer (270 mM, pH 4.0). Subsequently, 20 μL of ABTS (6 mM) and 5 μL of V₂O₃-OMC (0.2 mg mL⁻¹) were added into the above solution. After 5 min, the absorbance at 414 nm was measured.

Assessment of practicability for the colorimetric detection of glucose

For specificity analysis, 2 mM of lactose, galactose, maltose, fructose, xylose, and sucrose were used instead of 2 mM of glucose. The process of detection was same as the standard glucose assay. For analysis of real samples, the above detection method was used to detect glucose in real samples (peach juice and human serum). Prior to detection, the samples were diluted with PBS to fit into the linear range of the method.

Results and discussion

Characterization of V₂O₃-OMC

To study the morphology of V₂O₃-OMC, TEM was conducted for OMC and V₂O₃-OMC. The OMC exhibited the uniformly ordered arrangement of mesoporous channels (Fig. 1A). For the V₂O₃-OMC, the mesoporous structure of OMC remained intact, and some V₂O₃ nanoparticles homogeneously anchored in the channels of the OMC (Fig. 1B).

To confirm the formation of V₂O₃, XRD patterns of OMC, V₂O₃-OMC and the standard values of V₂O₃ (JCPDS 074-0325) were obtained (Fig. S1, ESI†). For V₂O₃-OMC, there were two broad diffraction peaks at 2θ of 23° and 43° in the XRD spectrum, which were consistent with OMC, indicating the existence of the OMC component. Meanwhile, all other diffraction peaks were consistent with the standard spectrum of V₂O₃ and no other impurities could be detected, suggesting that the pure V₂O₃ had been synthesized on the OMC. Furthermore, the Bragg peaks were mathematically analyzed and the V₂O₃ particle size was calculated to be about 47 nm by the Scherrer formula.

To determine the amount of V₂O₃ in V₂O₃-OMC, TGA of OMC, V₂O₃ and V₂O₃-OMC was carried out in air (Fig. S2, ESI†). For V₂O₃-OMC, the weight slightly declined below 300 °C, due to the evaporation of adsorbed moisture on the relatively high surface area of the materials. As the temperature rose successively toward 700 °C, the V₂O₃ was oxidized to V₂O₅ and the OMC was combusted. Therefore, the V₂O₃ content in the V₂O₃-OMC was calculated to be about 72 wt%.

Discovery of the peroxidase-like activity

Typically, the reactions were carried out using ABTS and H₂O₂ as the substrates in acetate buffer (pH 4.0) for 10 min. ABTS is a kind of common chromogenic substrate and is generally used for a typical GOx-peroxidase detection method and practical analytical application. To verify the peroxidase-like activity, the V₂O₃-OMC catalytic oxidation of ABTS and a series of control experiments were colorimetrically conducted from 370 to 500 nm (Fig. 2A). In the absence of V₂O₃-OMC (curve c), the reaction was slow, and the maximal absorption peak occurred at 414 nm, which was related to the oxidized ABTS (ABTS⁺). When OMC was added, the absorbance slightly increased (curve b), indicating that OMC slightly promoted the oxidation of ABTS. This could be because OMC also had weak peroxidase-like activity, just like other carbon materials. In the presence of V₂O₃-OMC (curve a), the absorbance at 414 nm was about three times the value in the absence of V₂O₃-OMC (curve c). To eliminate the possibility that the catalytic activity resulted from the leaching of free vanadium ions in acidic solution, V₂O₃-OMC was incubated in acetate buffer (pH 4.0) for 10 min, and then the V₂O₃-OMC was removed by centrifugation (14 000 rpm, 2 min). When the leaching solution was used instead of V₂O₃-OMC, the absorption did not change (curve d), compared with the case in the absence of V₂O₃-OMC (curve c), indicating that free vanadium ions did not have any catalytic activity. Taken together, due to the small size effect and large surface area, V₂O₃-OMC exhibited excellent peroxidase-like activity towards ABTS oxidation by H₂O₂. On visual observation, the reaction solution was much bluer in the presence of V₂O₃-OMC than other control experiments (inset of Fig. 2A), which also suggests the good catalytic performance of V₂O₃-OMC. However, the catalytic activity of V₂O₃ and its nanomaterials had rarely been investigated in contrast with V₂O₅ and VO₂. Therefore, our discovery was surprising. Moreover, the catalytic reaction rate was dependent on the amount of V₂O₃-OMC (Fig. 2B), further confirming the peroxidase-like activity of V₂O₃-OMC. So, we selected the mild concentration of V₂O₃-OMC (5 μg mL⁻¹) for subsequent analysis, where the absorbance at 414 nm not only increased linearly with time but also maintained the relatively higher value. In addition, the catalytic activity of bulk V₂O₃ was not observed under the same conditions (data not shown), indicating the importance of the nano-structure and heterogeneous composite for the peroxidase-like activity. Considering the electronegativity of ABTS, positively charged TMB, another typical chromogenic substrate,
was used for a comparison study with ABTS. After incubation with V$_2$O$_3$-OMC and H$_2$O$_2$ for 10 min, the TMB solution turned blue (Fig. S3, ESI†) with the maximum absorption at 652 nm, suggesting that V$_2$O$_3$-OMC could also quickly catalyze the oxidation of TMB. Therefore, V$_2$O$_3$-OMC is capable of catalyzing both ABTS and TMB, similar to HRP and other mimetics.37,59,60

To check the stability of V$_2$O$_3$ under H$_2$O$_2$ exposure, the XRD patterns of V$_2$O$_3$-OMC before and after 30 min of H$_2$O$_2$ (10 mM) exposure were recorded. The diffraction peaks of V$_2$O$_3$-OMC had obvious changes after H$_2$O$_2$ exposure (Fig. 3) and there was no formation of V$_2$O$_5$ (JCPDS 089-0612). The result showed that V$_2$O$_3$-OMC was stable under H$_2$O$_2$ exposure and the actual catalyst was V$_2$O$_3$, rather than V$_2$O$_5$.

Characterization of the peroxidase-like activity of V$_2$O$_3$-OMC

The effects of pH and temperature on the peroxidase-like activity of V$_2$O$_3$-OMC were studied by varying the pH from 1 to 12 and temperature from 15 to 60 °C while the concentrations of V$_2$O$_3$-OMC, ABTS and H$_2$O$_2$ were kept unchanged. It is noteworthy that the optimization curve had a sharp peak at pH 4.0, and the absorbance would decrease remarkably at more acidic or alkaline pH, indicating that the oxidation of ABTS catalyzed by the V$_2$O$_3$-OMC only occurred under weakly acidic conditions (Fig. 4A). For the temperature-dependent activity, the pH 4.0 buffer was applied. The enzyme activity slightly increased as the temperature increased until 40 °C, and thereafter, the activity decreased tremendously (Fig. 4B), probably because higher temperature could reduce the activity of V$_2$O$_3$.36,61 Therefore, like peroxidase, V$_2$O$_3$-OMC exhibited pH- and temperature-dependent activities. The optimal pH (pH 4.0) and temperature (40 °C) were chosen for subsequent experiments.

The V$_2$O$_3$-OMC remained over 90% relative activity in a wide range of pH from 1.0 to 12.0 (Fig. 4C), suggesting its high pH stability. On the other hand, V$_2$O$_3$-OMC was stable at temperatures within 4–40 °C. The activity declined dramatically upon the further increase in the temperature, and nevertheless, about 50% of the initial activity at 80 °C could still be retained (Fig. 4D). Therefore, V$_2$O$_3$-OMC showed good temperature stability. By contrast, HRP loses all activity at pH ≤ 5 or at temperature ≥70 °C under the similar conditions.34 Thus, it is apparent that inorganic V$_2$O$_3$-OMC was more stable than natural peroxidases.

Kinetics of catalytic reaction

The steady-state kinetics of the peroxidase mimetics was studied under the optimal conditions (pH 4.0 and 40 °C). To reasonably evaluate the catalytic performance of the V$_2$O$_3$-OMC, we detected the apparent kinetics parameters by
Michaelis–Menten kinetics (Fig. 5A and B) and obtained a $V_{\text{max}}$ of 0.29 $\mu$M$^{-1}$, $K_M$ (ABTS) of 0.067 mM and the $K_M$ (H$_2$O$_2$) of 0.16 mM. Thus the $K_M$ (ABTS) for V$_2$O$_3$-OMC was similar to that of SBP (0.045 mM),$^{62}$ and lower than those values of HRP (0.270 mM)$^{63}$ and PEI-coated Fe$_3$O$_4$ (0.12 mM),$^{64}$ suggesting that the V$_2$O$_3$-OMC had similar affinity for ABTS with natural enzymes. The $K_M$ (H$_2$O$_2$) of V$_2$O$_3$-OMC was similar to BSA-templated MnO$_2$ nanoparticles (0.12 mM) and higher than Fe$_3$O$_4$ nanoparticles (154 mM),$^{34}$ suggesting that H$_2$O$_2$ had high affinity for the V$_2$O$_3$-OMC. In addition, the $k_{\text{cat}}$ was calculated to be $1.28 \times 10^4$ s$^{-1}$, which was 5.12 times the value for V$_2$O$_5$ nanowires ($2.5 \times 10^3$ s$^{-1}$),$^{37}$ 4.66 times the value for SBP ($2.66 \times 10^3$ s$^{-1}$),$^{62}$ and 1.58 times the value for HRP ($8.1 \times 10^3$ s$^{-1}$),$^{63}$ indicating the high turnover rate of catalysis of V$_2$O$_3$-OMC. Further, the catalytic efficiency ($k_{\text{cat}}/K_M$) towards oxidation of ABTS was $1.91 \times 10^5$ $\mu$M$^{-1}$ s$^{-1}$, which was ca. 71 times higher than the value for SBP ($2.66 \times 10^3$ s$^{-1}$) and ca. 235 times higher than the values for HRP ($8.1 \times 10^2$ s$^{-1}$).$^{62,63}$ The excellent catalytic efficiency of V$_2$O$_3$-OMC could be originating from the following reasons. First and most importantly, OMC served as the supporter of V$_2$O$_3$ nanoparticles and made V$_2$O$_3$ nanoparticles homogeneously dispersed in the channels of the OMC. Due to the small size effect and large surface area, the nano-structural V$_2$O$_3$ had high catalytic activity. However, it was hard to synthesize nano-structural V$_2$O$_3$ by the traditional hydrogen reduction route.$^{48}$ Second, the OMC also had weak peroxidase-like activity. This was consistent with the experimental result that OMC slightly promoted the oxidation of ABTS (Fig. 2A, curve b).

To further investigate the kinetics mechanism, Lineweaver–Burk plots were obtained (Fig. 5C and D). The lines were nearly parallel to each other and the slopes were equal in each Lineweaver–Burk plot, which accords with the Ping-pong BiBi mechanism. That is to say, the V$_2$O$_3$-OMC interacted with the first substrate (H$_2$O$_2$) and released the first product before it reacted with the second substrate (ABTS), which is similar to HRP$^{65}$ and Fe$_3$O$_4$ nanoparticles.$^{34}$ It is noteworthy that the catalytic mechanism of V$_2$O$_3$-OMC was different from that of another vanadium oxide, V$_2$O$_5$, where H$_2$O$_2$ and ABTS were bound orderly to V$_2$O$_5$, and then the ABTS$^+$ was subsequently released.$^{37}$ This discrepancy could be caused by the different vanadium coordination geometries between V$_2$O$_3$ and V$_2$O$_5$ lattice planes.$^{37}$ However, judging from EPR spectra for V$_2$O$_3$ (Fig. S4, ESI†), the generation of superoxides and hydroxyl radicals could not be identified, which was different from V$_2$O$_5$.$^{37}$ A symmetrical and sharp signal peak appeared at around 3500 G for the mixture of V$_2$O$_3$ + H$_2$O$_2$, which was not observed for either H$_2$O$_2$ or aqueous V$_2$O$_3$ suspension. This signal could be attributed to an electron trapped on an oxygen vacancy or unknown free radicals in the crystal lattice of V$_2$O$_3$ after H$_2$O$_2$ treatment. The phenomenon could make some contribution to the oxidation of ABTS, although the exact mechanism was unclear for the moment.
Colorimetric detection of H$_2$O$_2$

At the optimal pH (pH 4.0) and room temperature (25 °C), the colorimetric detection of H$_2$O$_2$ was conveniently carried out using the V$_2$O$_3$-OMC as the catalyst. The calibration curve is plotted based on the absorbance at 414 nm as a function of H$_2$O$_2$ concentration (Fig. S5, ESI†). The linear range was 0.005–0.25 mM with the regression equation of $y = 2.4169x + 0.2001$ ($R^2 = 0.996$). The linear range was wider than that of the Fe$_3$O$_4$ MNP- (0.005–0.1 mM)$^{35}$ and Au NP- (0.002–0.2 mM)$^{66}$ based colorimetric detection of H$_2$O$_2$. The limit of detection (LOD) in our case was 1.7 μM, which was lower than that of Fe$_3$O$_4$ MNPs (3.0 μM)$^{35}$.

Colorimetric detection of glucose and assessment of practicability

The colorimetric detection of glucose with a simple procedure, short analysis time, small sample volume and low cost could be conveniently realized by using GOx and V$_2$O$_3$-OMC. Because GOx would be denatured in pH 4.0 buffer, the detection of glucose was conducted in two separated and coupled steps: oxidation of glucose and detection of H$_2$O$_2$. The linear range was 0.01–4 mM glucose with the regression equation of $y = 0.2838x + 0.0043$ ($R^2 = 0.998$) (Fig. 6), which was wider than the Fe$_3$O$_4$ MNP- (0.05–1 mM)$^{67}$ and Au NP- (0.018–1.1 mM)$^{66}$ based colorimetric detection of glucose. The LOD was 3.3 μM glucose, which was lower than that of Fe$_3$O$_4$ MNPs (30 μM)$^{35}$ and Au NPs (4 μM)$^{66}$.

To investigate the specificity of this method, a control experiment was conducted using other sugars instead of glucose (Fig. S6, ESI†). Because 2 mM glucose was within the linear detection range of 0.01–4 mM, we selected the same concentration (2 mM) of other sugars for the specificity test.
Due to the specificity of GOx, the method had no obvious responses to the control sugars and showed the excellent specificity towards the detection of glucose. In addition, the influence of NaCl on the detection of glucose was also investigated, since chloride ions are abundant in nature and can affect the activity of inorganic nanomaterials. There was no obvious difference between the absorbance for 2 mM glucose in the presence of 0.15 M NaCl and the absorbance in the absence of NaCl (Fig. S6, ES1†), indicating that the method could be reliable for the real sample (such as serum) containing plenty of chloride ions.

To evaluate the reproducibility of the proposed method, 5 aliquots of glucose solutions (2 mM) were separately detected. The relative standard deviation was 4.3%, indicating high precision and acceptable reproducibility of this method. In addition, no obvious change in the catalytic activity of the V2O3-OMC was observed after 30 days of storage at room temperature, suggesting the excellent storage stability of V2O3-OMC.

For batch-to-batch reproducibility of the as-prepared V2O3-OMC, three batches of V2O3-OMC products were independently used for the detection of 2 mM glucose. The relative standard deviation (RSD) was 4.7%, demonstrating the excellent batch-to-batch reproducibility of the as-synthesized V2O3-OMC.

To confirm the practicability, the method was used for the analysis of real samples. The concentrations were calculated based on the calibration curve (Table 1). The results by our method consisted well with the known results by conventional GOx-HRP colorimetric methods and the RSDs were within 3.2%. The recoveries were 98.2–102.9%, demonstrating the excellent precision, accuracy and reliability of the proposed method for the practical application.

Conclusions

In summary, V2O3-OMC was synthesized and studied as a peroxidase mimetic for the first time. The as-prepared V2O3-OMC had high intrinsic catalytic activity towards typical peroxidase substrates (H2O2 and ABTS). Like natural peroxidase, the activity of V2O3-OMC was dependent on pH and temperature. The kinetics parameters and probable kinetics mechanisms were investigated. The V2O3-OMC achieved the significantly highest value among the kcat values reported so far. Furthermore, we developed a colorimetric method for the detection of H2O2 by using V2O3-OMC, and a coupled method for the detection of glucose by using V2O3-OMC and GOx, which showed high sensitivity, wide detection range, excellent selectivity and good reliability for real samples. Due to its easy preparation, high activity, good stability and excellent analytical performance, V2O3-OMC would open up a potential avenue in the catalytic industry, bioassay and environmental science.

Acknowledgements

The authors are grateful for the financial support from the National Natural Science Foundation of China (no. 91227116, 21275152 and 21475144).

Notes and references


Table 1 Detection of glucose in real samples based on the proposed method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Known conc. a (mM)</th>
<th>Detected conc. b (mM)</th>
<th>RSD</th>
<th>Added (mM)</th>
<th>Found c (mM)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 a</td>
<td>10.91</td>
<td>10.57 ± 0.31</td>
<td>-3.2%</td>
<td>1.00</td>
<td>11.55 ± 0.42</td>
<td>98.2%</td>
</tr>
<tr>
<td>2 b</td>
<td>0.379</td>
<td>0.381 ± 0.02</td>
<td>+0.5%</td>
<td>1.00</td>
<td>1.41 ± 0.06</td>
<td>102.9%</td>
</tr>
<tr>
<td>3 c</td>
<td>4.03</td>
<td>4.12 ± 0.09</td>
<td>+2.2%</td>
<td>1.00</td>
<td>5.13 ± 0.11</td>
<td>101.0%</td>
</tr>
</tbody>
</table>

a Human serum sample from a local hospital. b Local peach juice. c The reaction solution of straw degraded by cellulase. d All values were expressed as the mean value plus-minus the standard deviation for three repetitive experiments.