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

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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-ethylbenzo-thiazoline-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 $1.28 \times 10^4 \text{ s}^{-1}$, 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 mimetic holds great promise for broad potential applications.

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Introduction

Enzymes are of great scientific and practical significance in the fields of clinical diagnosis, chemical industry, environmental protection and food production,^{1–3} 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.^{4–7} 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,^{8–10} metals,^{11–13} and metal-oxides,^{14,15} have widely been used as mimetic enzymes.¹⁶

So far, many enzymes have corresponding mimetics, such as glycosidase,^{17,18} phosphatase,¹⁹ epoxidase,²⁰ protease,^{21,22} nuclease,²² oxidase,²³ peroxidase^{8,24} and superoxide dismutase.²⁵ Peroxidase is one of the important enzymes and has been widely applied to chemical analysis, medical diagnosis and wastewater treatment.^{26–28} 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 compounds²⁹ and their oxidation can reduce their toxicity or provide a color change, which is detectable by colorimetric methods.³⁰ 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.^{31,32}

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,³³ such as Fe_3O_4 magnetic nano-

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particles,^{14,34,35} bovine serum albumin (BSA)-templated MnO₂ nanoparticles,³⁶ V₂O₅ nanowires,³⁷ and Co₃O₄ nanoparticles.³⁸ Vanadium is a transition metal element and has been found in some organisms such as algae and fungi.³⁹ 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.^{39,40} Vanadium oxides, mainly including V₂O₅, VO₂ and V₂O₃,⁴¹ have been extensively studied for their electro-optic effect,⁴² electrochromic properties,^{43,44} field emission,⁴⁵ and their use in lithium batteries,^{46–48} catalysts,^{37,49} electrochemical ethanol sensors,^{50,51} photocatalysis,⁵² thermochromic smart windows⁵³ and surface-enhanced Raman scattering.⁵⁴ 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.⁴⁸ 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^{•+}).³⁵ 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 ($\epsilon_{414\text{ nm}} = 36\text{ mM}^{-1}\text{ cm}^{-1}$)⁵⁵ 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 K_m and V_{max} were calculated by fitting the data to the Michaelis-Menten equation: $v = V_{max} \times [S]/(K_m + [S])$, where v is the reaction velocity, V_{max} is the maximal reaction velocity, $[S]$ is the concentration of the substrate and K_m is the Michaelis constant. The turnover frequency (k_{cat} , also called catalytic constant) is calculated using the formula: $k_{cat} = V_{max}/[V_2O_3-OMC]$. 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 ($\text{glucose} + \text{O}_2 \rightarrow \text{H}_2\text{O}_2 + \text{gluconic acid}$) was coupled with the colorimetric detection of H_2O_2 . 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 acetate buffer (270 mM, pH 4.0). Subsequently, 20 μL of ABTS (6 mM) and 5 μL of V_2O_3 -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 V_2O_3 -OMC

To study the morphology of V_2O_3 -OMC, TEM was conducted for OMC and V_2O_3 -OMC. The OMC exhibited the uniformly ordered arrangement of mesoporous channels (Fig. 1A). For the V_2O_3 -OMC, the mesoporous structure of OMC remained intact, and some V_2O_3 nanoparticles homogeneously anchored in the channels of the OMC (Fig. 1B).

To confirm the formation of V_2O_3 , XRD patterns of OMC, V_2O_3 -OMC and the standard values of V_2O_3 (JCPDS 074-0325) were obtained (Fig. S1, ESI†). For V_2O_3 -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 V_2O_3 and no other impurities could be detected, suggesting that the pure V_2O_3 had been synthesized on the OMC. Furthermore, the

Bragg peaks were mathematically analyzed and the V_2O_3 particle size was calculated to be about 47 nm by the Scherrer formula.

To determine the amount of V_2O_3 in V_2O_3 -OMC, TGA of OMC, V_2O_3 and V_2O_3 -OMC was carried out in air (Fig. S2, ESI†). For V_2O_3 -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 V_2O_3 was oxidized to V_2O_5 and the OMC was combusted. Therefore, the V_2O_3 content in the V_2O_3 -OMC was calculated to be about 72 wt%.

Discovery of the peroxidase-like activity

Typically, the reactions were carried out using ABTS and H_2O_2 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,⁵⁶ and practical analytical application.⁵⁷ To verify the peroxidase-like activity, the V_2O_3 -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 V_2O_3 -OMC (curve c), the reaction was slow, and the maximal absorption peak occurred at 414 nm, which was related to the oxidized ABTS (ABTS^*).⁵⁵ 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.^{9,58} In the presence of V_2O_3 -OMC (curve a), the absorbance at 414 nm was about three times the value in the absence of V_2O_3 -OMC (curve c). To eliminate the possibility that the catalytic activity resulted from the leaching of free vanadium ions in acidic solution, V_2O_3 -OMC was incubated in acetate buffer (pH 4.0) for 10 min, and then the V_2O_3 -OMC was removed by centrifugation (14 000 rpm, 2 min). When the leaching solution was used instead of V_2O_3 -OMC, the absorption did not change (curve d), compared with the case in the absence of V_2O_3 -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, V_2O_3 -OMC exhibited excellent peroxidase-like activity towards ABTS oxidation by H_2O_2 . On visual observation, the reaction solution was much bluer in the presence of V_2O_3 -OMC than other control experiments (inset of Fig. 2A), which also suggests the good catalytic performance of V_2O_3 -OMC. However, the catalytic activity of V_2O_3 and its nano-materials had rarely been investigated in contrast with V_2O_5 and VO_2 . Therefore, our discovery was surprising. Moreover, the catalytic reaction rate was dependent on the amount of V_2O_3 -OMC (Fig. 2B), further confirming the peroxidase-like activity of V_2O_3 -OMC. So, we selected the mild concentration of V_2O_3 -OMC (5 $\mu\text{g mL}^{-1}$) 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 V_2O_3 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,

Fig. 1 TEM images of (A) OMC and (B) V_2O_3 -OMC.

Fig. 2 (A) Typical absorption curves of reaction solutions catalyzed by the as-prepared V_2O_3 -OMC for 10 min in 0.6 mM ABTS buffered with acetate solution (100 mM, pH 4.0) containing (a) H_2O_2 (1 mM) and V_2O_3 -OMC ($5 \mu g mL^{-1}$); (b) H_2O_2 (1 mM) and OMC ($5 \mu g mL^{-1}$); (c) H_2O_2 (1 mM); (d) H_2O_2 (1 mM) and leaching of vanadium ions; (e) V_2O_3 -OMC ($5 \mu g mL^{-1}$). Inset, the corresponding photographs of reaction solutions in microplates. (B) Dependency of peroxidase-like activity on the V_2O_3 -OMC concentration. The absorbance was monitored at 414 nm, in 200 μL acetate buffer (0.2 M, pH 4.0) containing 0.6 mM ABTS and 1 mM H_2O_2 with varying amounts of V_2O_3 -OMC at 37 $^{\circ}C$: 0 (a), 0.25 (b), 0.5 (c), 1 (d), 1.5 (e), 2 μg (f).

was used for a comparison study with ABTS. After incubation with V_2O_3 -OMC and H_2O_2 for 10 min, the TMB solution turned blue (Fig. S3, ESI †) with the maximum absorption at 652 nm, suggesting that V_2O_3 -OMC could also quickly catalyze the oxidation of TMB. Therefore, V_2O_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_2O_3 under H_2O_2 exposure, the XRD patterns of V_2O_3 -OMC before and after 30 min of H_2O_2 (10 mM) exposure were recorded. The diffraction peaks of V_2O_3 -OMC had obvious changes after H_2O_2 exposure (Fig. 3) and there was no formation of V_2O_5 , according to the standard values of V_2O_5 (JCPDS 089-0612). The result showed that V_2O_3 -OMC was stable under H_2O_2 exposure and the actual catalyst was V_2O_3 rather than V_2O_5 .

Characterization of the peroxidase-like activity of V_2O_3 -OMC

The effects of pH and temperature on the peroxidase-like activity of V_2O_3 -OMC were studied by varying the pH from 1 to 12 and temperature from 15 to 60 $^{\circ}C$ while the concentrations of V_2O_3 -OMC, ABTS and H_2O_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_2O_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 $^{\circ}C$, and thereafter, the activity decreased tremendously (Fig. 4B), probably because higher temperature could reduce the activity of V_2O_3 .^{36,61} Therefore, like peroxidase, V_2O_3 -OMC exhibited pH- and temperature-dependent activities. The optimal pH (pH 4.0) and temperature (40 $^{\circ}C$) were chosen for subsequent experiments.

The V_2O_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_2O_3 -OMC was stable at temperatures within 4–40 $^{\circ}C$. The activity declined dramatically upon the further increase in the temperature, and nevertheless, about 50% of the initial activity at 80 $^{\circ}C$ could still be retained (Fig. 4D). Therefore, V_2O_3 -OMC showed good temperature stability. By contrast, HRP loses all activity at pH ≤ 5 or at temperature ≥ 70 $^{\circ}C$ under the similar conditions.³⁴ Thus, it is apparent that inorganic V_2O_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 $^{\circ}C$). To reasonably evaluate the catalytic performance of the V_2O_3 -OMC, we detected the apparent kinetics parameters by

Fig. 3 The XRD patterns of V_2O_3 -OMC (a) before and (b) after 30 min of H_2O_2 (10 mM) exposure, and (c) bulk V_2O_3 .

Fig. 4 The optimization of pH (A) and temperature (B) for the peroxidase-like activity of the V₂O₃-OMC. The pH stability (C) and temperature stability of V₂O₃-OMC (D). For each curve, the highest point was defined as 100% relative activity.

Michaelis-Menten kinetics (Fig. 5A and B) and obtained a $V_{\max}$ of $0.29 \mu\text{M s}^{-1}$, K_M (ABTS) of 0.067 mM and the K_M (H_2O_2) of 0.16 mM . Thus the K_M (ABTS) for V₂O₃-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 Fe₃O₄ (0.12 mM),⁶⁴ suggesting that the V₂O₃-OMC had similar affinity for ABTS with natural enzymes. The K_M (H_2O_2) of V₂O₃-OMC was similar to BSA-templated MnO₂ nanoparticles (0.12 mM) and higher than Fe₃O₄ nanoparticles (154 mM),³⁴ suggesting that H_2O_2 had high affinity for the V₂O₃-OMC. In addition, the k_{cat} was calculated to be $1.28 \times 10^4 \text{ s}^{-1}$, which was 5.12 times the value for V₂O₅ nanowires ($2.5 \times 10^3 \text{ s}^{-1}$),³⁷ 4.66 times the value for SBP ($2.66 \times 10^3 \text{ s}^{-1}$)⁶² and 1.58 times the value for HRP ($8.1 \times 10^3 \text{ s}^{-1}$),⁶³ indicating the high turnover rate of catalysis of V₂O₃-OMC. Further, the catalytic efficiency (k_{cat}/K_M) towards oxidation of ABTS was $1.91 \times 10^5 \mu\text{M}^{-1} \text{ s}^{-1}$, which was *ca.* 71 times higher than the value for SBP ($2.66 \times 10^3 \text{ s}^{-1}$) and *ca.* 235 times higher than the values for HRP ($8.1 \times 10^2 \text{ s}^{-1}$).^{62,63} The excellent catalytic efficiency of V₂O₃-OMC could be originating from the following reasons. First and most importantly, OMC served as the supporter of V₂O₃ nanoparticles and made V₂O₃ nanoparticles homogeneously dispersed in the channels of the OMC. Due to the small size effect and large surface area, the nano-structural V₂O₃ had high catalytic activity. However, it was hard to synthesize nano-structural V₂O₃ by the traditional hydrogen reduction route.⁴⁸ Second, the OMC also had weak peroxidase-like activity. This was consistent with the experi-

mental 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 V₂O₃-OMC interacted with the first substrate (H_2O_2) and released the first product before it reacted with the second substrate (ABTS), which is similar to HRP⁶⁵ and Fe₃O₄ nanoparticles.³⁴ It is noteworthy that the catalytic mechanism of V₂O₃-OMC was different from that of another vanadium oxide, V₂O₅, where H_2O_2 and ABTS were bound orderly to V₂O₅, and then the ABTS^{•+} was subsequently released.³⁷ This discrepancy could be caused by the different vanadium coordination geometries between V₂O₃ and V₂O₅ lattice planes.³⁷ However, judging from EPR spectra for V₂O₃ (Fig. S4, ESI†), the generation of superoxides and hydroxyl radicals could not be identified, which was different from V₂O₅.³⁷ A symmetrical and sharp signal peak appeared at around 3500 G for the mixture of V₂O₃ + H_2O_2 , which was not observed for either H_2O_2 or aqueous V₂O₃ suspension. This signal could be attributed to an electron trapped on an oxygen vacancy or unknown free radicals in the crystal lattice of V₂O₃ after H_2O_2 treatment. The phenomenon could make some contribution to the oxidation of ABTS, although the exact mechanism was unclear for the moment.

Fig. 5 The steady-state kinetics analysis. Michaelis–Menten curves from activity data of the fixed concentration of ABTS (0.6 mM) and various concentrations of H_2O_2 (0.01–10 mM) (A), and the fixed concentration of H_2O_2 (10 mM) and various concentrations of ABTS (0.001–0.6 mM) (B). The Lineweaver–Burk plots from activity data of the concentrations of H_2O_2 (0.5, 1 and 2 mM) (C) and ABTS (0.03, 0.06 and 0.12 mM) were varied one at a time (D).

Colorimetric detection of H_2O_2

At the optimal pH (pH 4.0) and room temperature (25 °C), the colorimetric detection of H_2O_2 was conveniently carried out using the V_2O_5 -OMC as the catalyst. The calibration curve is plotted based on the absorbance at 414 nm as a function of H_2O_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_3O_4 MNP- (0.005–0.1 mM)³⁵ and Au NP- (0.002–0.2 mM)⁶⁶ based colorimetric detection of H_2O_2 . The limit of detection (LOD) in our case was 1.7 μM , which was lower than that of Fe_3O_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_2O_5 -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_2O_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_3O_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_3O_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.

Fig. 6 The calibration curve for glucose.

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

Sample	Known conc. ^d (mM)	Detected conc. ^e (mM)	RSD	Added (mM)	Found ^e (mM)	Recovery
1# ^a	10.91	10.57 ± 0.31	−3.2%	1.00	11.55 ± 0.42	98.2%
2# ^b	0.379	0.381 ± 0.02	+0.5%	1.00	1.41 ± 0.06	102.9%
3# ^c	4.03	4.12 ± 0.09	+2.2%	1.00	5.13 ± 0.11	101.0%

^a Human serum sample from a local hospital. ^b Local peach juice. ^c The reaction solution of straw degraded by cellulase. ^d The known concentration of glucose was detected by the GOx-HRP colorimetric method. ^e All values were expressed as the mean value plus-minus the standard deviation for three repetitive experiments.

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.⁶⁸ 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, ESI†), 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 *k*_{cat} 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.

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