Single-layer MnO₂ nanosheets for sensitive and selective detection of glutathione by a colorimetric method

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ABSTRACT

The rapid, sensitive and selective detection of glutathione (GSH) is of great importance in the biological systems. In this work, a template-free and one-step method was used to synthesize the single-layer MnO₂ nanosheets via a redox reaction. The resulting product was characterized by XRD, TEM, FTIR, XPS and UV-vis absorption. The addition of GSH results in the change of solution color depth owing to the occurrence of a redox reaction between MnO₂ and GSH, enabling colorimetric detection of GSH. At a pH of 3.6, the proposed sensor gives a linear calibration over a GSH concentration range of 10 to 100 μ M, with a rapid response of less than 2 min and a low detection limit of 0.5 μ M. The relative standard deviation for seven repeated determinations of GSH is lower than 5.6%. Furthermore, the chemical response of the synthesized MnO₂ nanosheets toward GSH is selective. Owing to the advantages with good water solubility, rapid response, high sensitivity, good biocompatibility and operation simplicity, this two-dimensional MnO₂-based sensing material might be potential for detecting GSH in biological applications.

Keywords: Manganese dioxide; Single-layer nanosheets; Glutathione detection; Colorimetric method; Redox reaction

1. Introduction

Glutathione (GSH) is the most abundant cellular biothiols and essential endogenous antioxidants. It plays a central role in cellular defense against toxins and free radicals [1,2]. Abnormal levels of GSH has been implicated with the development of many kinds of diseases. For example, GSH deficiency can lead to various diseases such as leukocyte loss, cancer, AIDS and neurodegenerative disease [3–6]. Therefore, the rapid, convenient, sensitive and selective detection of GSH is of great importance in clinical diagnosis [4–7].

Nanomaterials are potential candidates for analyte detection, because their reduced size leads to an exceptionally high surface area and tunable surface chemistry, and thus creates a remarkable increase in sensor sensitivity towards change in its surrounding chemical environment [8,9]. Nanosheets are a class of two-dimensional (2D) nanomaterials with high specific surface area, characterized by a thickness of nanometers and lateral dimensions of sub-micrometers to micrometers. Recently, the emerging (2D) nanomaterials (e.g., graphene, MoS₂, WS₂, etc.) have attracted wide attention due to their unique physicochemical properties, and found the potential applications in a variety of areas including electronics, energy storage, catalysis, and biology [10–16]. Particularly, for single-layer 2D nanomaterials, an intense interest has been focused to develop the intelligent sensing nanosystems that can interact with various biomolecules and respond rapidly with their electrical, optical or magnetic properties for biodetection [17–22].

Manganese dioxide (MnO₂) is a well-known functional transition metal oxide. Owing to its unique physicochemical properties, and environmental and biological compatibility, MnO₂ has attracted wide research interest and found diverse potential applications including cell batteries [23,24], catalyst [25–28], sensing [29,30], and biological uses in the magnetic resonance imaging and drug delivery [31,32].

In this work, we used a template-free and one-step method to synthesize single-layer MnO₂ nanosheets via a redox reaction between KMnO₄ and sodium dodecyl sulfate. The synthesized MnO₂ nanosheets can be dispersed in the water to

form the stable brown colloidal solution with an intense absorption in the UV-vis region centered at 375 nm. The addition of GSH to MnO_2 solution at an acidic pH condition led to a quick transformation of the solution color from brown to colorlessness due to the reduction of MnO_2 to Mn(II) by GSH, which enabled the colorimetric determination of GSH.

2. Experimental

2.1. Chemicals

Analytical grade KMnO_4 , sodium dodecyl sulfate (SDS), concentrated sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), citric acid and sodium citrate were obtained from Beijing Chemicals Reagents. GSH, glucose, fructose, uric acid, cysteine (Cys) and 1,4-dithiothreitol (DTT) were purchased from Sigma-Aldrich Co. (Shanghai, China). MilliQ water was used throughout. All other chemical reagents were of analytical reagent grade. The citrate buffer was prepared by mixing an approximate ratio of citric acid and sodium citrate solutions.

2.2. Synthesis of Single-Layer MnO_2 Nanosheets

Single-layer MnO_2 nanosheets were synthesized following a typical process as below [33]. 32 mL of SDS solution (0.1 M) and 1.6 mL of H_2SO_4 solution (0.1 M) were added into 283.2 mL distilled water and heated at 95 °C for 15 min first. 3.2 mL of KMnO_4 solution (0.05 M) was added into the above solution quickly to start the reaction and the reaction mixture was maintained at 95 °C for 60 min. In this process, the initial KMnO_4 solution with purplish red color was gradually transformed to the dark brown colloidal suspension. The resulting suspensions were centrifuged, and the precipitates were thoroughly washed with ethanol for 3 times and subsequently dried in air at 50 °C for various analyses. The purified MnO_2 was dispersed in citrate buffer to form a colloidal suspension for analyte detection.

2.3. GSH detection

In a typical process of GSH detection, 50 μL of GSH solution with a given concentration was added into 2 mL of citrate buffer containing MnO_2 (0.05 mM). The above mixed solution was reacted with a slight shaking for 3 min. Then, the

supernatant was immediately moved to a quartz cuvette for UV-vis absorption measurement. To establish the relationship between the absorbance of the solution and GSH concentration, GSH concentration was changed, but other reaction conditions keep the same according to the above experiment procedure.

2.4. Characterizations

The X-ray powder diffraction (XRD) data were collected on an X'Pert MPD Philips diffractometer (CuK α X-radiation at 40 kV and 50 mA) with a scanning step of 0.02°. The transmission electron microscopy (TEM) observations were carried out using a JEOL 2200FS microscope. Samples for TEM investigations were prepared by first dispersing the particles in ethanol under assistance of ultrasonification and then dropping one drop of the suspension on a copper TEM grid coated with a holey carbon film. Fourier transform infrared (FT-IR) spectra (Mattson 5000) of the samples were measured in the range of 4000-450 cm⁻¹ in transmission mode. The pellets were prepared by adding 0.8 mg of the sample powder to 80 mg of KBr. The powders were mixed homogeneously and compressed at a pressure of 10 KPa to form transparent pellets. X-ray photoelectron spectroscopy (XPS) analysis was performed using a PHI Quantera SXM (ULVAC-PHI) device operating at a pressure of 10⁻⁸ Torr. The photoelectron emission spectra were recorded using a monochromatic Al K α source (100 W). The angle between the x-ray direction and the emitted electron direction was 45°. The UV-vis absorbance measurements were carried out using a Shimadzu UV-2550 scanning spectrophotometer with a scan rate of 240 nm min⁻¹. For the measurement of the absorbance evolution with time after adding a given concentration of GSH, an immediate measurement was carried out, and a fast scan rate of 1200 nm min⁻¹ was used.

3. Results and discussion

3.1. Synthesis and characterization of Single-layer MnO₂ nanosheets

A template-free and one-step method was used to synthesize the single-layer MnO₂ nanosheets via a redox reaction between KMnO₄ and sodium dodecyl sulfate (SDS). The crystal structure of the synthesized product was characterized by XRD.

The XRD profile shown in Fig. 1(a) exhibited four characteristic peaks at $2\theta = 12.1^\circ, 24.2^\circ, 36.7^\circ, 66^\circ$, indicating a typical lamellar structure. All the diffraction peaks can be well indexed to δ - MnO_2 phase (JCPDS No. 18-0802). Fig. 1(b) shows a TEM image of representative areas of the as-synthesized product. The sample was typically composed of ultrathin and transparent lamellar structure with ample graphene-like wrinkles and folds, displaying a typical 2D morphology of MnO_2 nanosheets [33]. The perceived average lateral dimension of the nanosheets is estimated to be ~ 200 nm. The thickness of nanosheets was characterized to be smaller than 1 nm, confirming the formation of single-layer MnO_2 nanosheets.

The FT-IR spectrum (Fig. 1(c)) provides further insight into the structure and surface state of synthesized MnO_2 . The peaks at $518, 473\text{ cm}^{-1}$ are assigned to the characteristic absorption of the Mn–O stretching vibration of octahedral $[\text{MnO}_6]$ framework [34,35]. Two intense bands at 3420 and 1625 cm^{-1} are attributed to the physically adsorbed water and the interlayer water in the MnO_2 nanosheets [34]. The low intensity of peaks in the $2922\text{--}2995\text{ cm}^{-1}$ region are assignable to the asymmetric and symmetric $-\text{CH}_2$ and $-\text{CH}_3$ stretching due to the use of SDS in the synthesis [36].

XPS was used to make a qualitative analysis of chemical valence and binding of the element for the synthesized MnO_2 . Two characteristic peaks centered at 642.2 and 654.1 eV were observed, corresponding to Mn $2p_{3/2}$ and Mn $2p_{1/2}$ of MnO_2 , respectively (Fig. 1(d)) [34,37]. The spin-energy separation of $\sim 11.9\text{ eV}$ is also consistent with those previous reports by other research groups [34,38]. No additional signals attributed to Mn_2O_3 and KMnO_4 were found in the XPS spectrum, indicating the generation of pure MnO_2 . The XPS spectrum of oxygen shows two peaks centered at 529.8 and 532.7 eV (Fig. 1(e)), which are assigned to the lattice oxygen of $[\text{MnO}_6]$ octahedra and the oxygen in the interlayer H_2O or H_3O^+ , in good agreement with the FTIR result [34].

The UV-vis absorption spectrum (Fig. 1(f)) of the synthesized MnO_2 solution exhibits a broad absorption band around $\sim 375\text{ nm}$, which is attributed to the d-d transition of Mn(IV) in the octahedral $[\text{MnO}_6]$ unit. The wavelength and intensity of

the absorbance are in line with the previous findings of single-layer MnO₂ nanosheets by other groups [39–41]. The colloidal suspension of single-layer MnO₂ nanosheets shows good stability and remains stable at 4 °C in the dark for more than 15 days without any precipitates.

Insert Fig. 1

3.2. Redox reaction between MnO₂ and GSH

In our preliminary experiment, it is interesting to find that, the addition of GSH to MnO₂ solution caused the MnO₂ solution to fade. Correspondingly, the UV-vis absorbance of the MnO₂ solution decreased due to the reaction of MnO₂ with GSH. Moreover, the reactivity of MnO₂ with GSH depended strongly on the solution pH values. To demonstrate the effect of solution pH on the reactivity of MnO₂ with GSH, the experiments were conducted as below. The equivalent quantities of MnO₂ were dispersed in 5 mL citrate buffer with different pH values ranging from 3.8 to 5.6 and MiliQ water with pH 7.4, respectively. Then, 50 µL of GSH solution (0.1 mM) was added and reacted at room temperature for 3 min. The supernatant was immediately moved to a quartz cuvette for UV-vis absorption measurement. The absorbance of MnO₂ solution after reaction as a function of pH values was recorded in Fig. 2. We can see that the absorbance of MnO₂ solution with the addition of GSH decreases rapidly with the decrease of pH value. For a parallel comparison, we also measured the UV-vis absorption spectra of MnO₂ solution under the different pH conditions, but without the addition of GSH. We can see that the absorbance of MnO₂ solution without the addition of GSH decreases slightly with the decrease of pH values ranging from 7.4 to 3.6 (the inset of Fig.2). In contrast, MnO₂ nanosheets seems stable at pH 7.4 in the absence of GSH. Even with GSH added, most of MnO₂ retains under this neutral condition. This indicates that an acidic reaction condition with low pH value benefits the reaction of MnO₂ nanosheets with GSH, and that the presence of GSH accelerates the degradation of MnO₂ in acidic solution.

In addition, the reaction speed was found to also depend on the pH value of reaction solution. The decrease of pH value facilitates the reaction between MnO₂ and

GSH (data not shown). The above results indicate that the acid reaction condition is necessary for the reaction of MnO₂ with GSH. Indeed, upon the addition of GSH to MnO₂ solution in the presence of H⁺, the redox reaction occurs following the equation (1), in which GSH was oxidized to generate glutathione disulfide (GSSG) and MnO₂ was reduced to Mn²⁺ [7,29]. Therefore, the reaction of MnO₂ with GSH in an acid aqueous solution might enable the colorimetric detection for GSH. In the following experiments, pH 3.6 was chosen for the reaction of MnO₂ with GSH.

Insert Fig. 2

3.3. Response time

The response rate of MnO₂ solution toward GSH at pH 3.6 was demonstrated by sequentially measuring the absorbance at a time interval of 30 s, which was recorded in Fig. 3. The absorbance of MnO₂ solution decreases immediately upon the addition GSH into MnO₂ solution, and reaches an almost unchanged value during a short time, less than 2 min. This indicates a quick reaction between MnO₂ with GSH, imparting the sensor with the function of a rapid detection. The rapid chemical response of MnO₂ nanosheets to analytes is attributed to the following two aspects. On one hand, the acidic pH 3.6 condition supplies enough H⁺ ions that facilitates the reaction of MnO₂ with GSH, as mentioned earlier (equation (1)); on the other hand, the ultrathin MnO₂ nanosheets possess a large surface-to-volume ratio that is expected to enhance the interaction of MnO₂ with GSH [42].

Insert Fig. 3

3.4 Sensitivity and reliability

Fig. 4(a) shows the UV-vis absorption spectra of MnO₂ solution after reaction upon the addition of various concentrations of GSH at pH 3.6. All UV-vis absorption spectra exhibited a broad band around 375 nm that originates from the d-d transition of Mn(IV) in the octahedral [MnO₆] unit. With an increase of GSH concentration, the

absorbance of the solution decreases gradually due to the reduction of MnO_2 to Mn^{2+} by GSH. Indeed, the variation of color depth of the solution as a result of the change of GSH concentration added can be observed directly by the naked eyes, as shown in the photographs taken for samples treated with various concentrations of GSH (Fig. 4(b)). With the increase of GSH concentration added, the solution faded gradually. The dependence of the absorbance on GSH concentration shows the feasibility of GSH determination by using MnO_2 /GSH reaction system. This colorimetric method provides a simple, low-cost and convenient assay for GSH since the synthesis of MnO_2 nanosheets is quite simple and this method does not need expensive instruments and complicated operations.

The sensing sensitivity of colloidal MnO_2 solution toward GSH was evaluated based on the relationship between the absorbance and analyte concentration. The absorbance at 375 nm relative to GSH concentration was collected in Fig. 4(c). Clearly, the synthesized MnO_2 solution was highly sensitive to GSH since the absorbance shows a rapid change as GSH concentration changes. Moreover, the absorption intensity exhibits a good linear relationship against GSH concentration in the range of 0.01–0.1 mM with a correlation coefficient $R^2 = 0.968$, indicating a wide linear response for GSH sensing. By a linear fitting, the regression equation for GSH was $A = 0.556 - 6300[\text{GSH}] \text{ (M)}$, where A represents the absorbance of the resulting solution at a given GSH concentration added. The detection limit (LOD) was defined by the equation $\text{LOD} = (3\sigma/s)$ at the signal-to-noise of 3, where σ is the standard deviation of the blank signals for 11 replicate detections and s is the slope of the calibration curve [43–46]. Based on the above calibration equation, the LOD for GSH was calculated to be 0.5 μM .

To verify whether our present MnO_2 nanosheets for GSH detection can work following this linear sensing function, we randomly selected two concentration points of GSH (15 and 45 μM) from this linear plot to measure their absorbance corresponding to these two GSH concentrations. Results show that the measured absorbance values fit well with the linear sensing function: the relative deviation of

absorbance for 15 and 45 μM GSH was 5.6% and 2.7%, respectively, which indicated acceptable precision.

Insert Fig. 4

3.5. Selectivity

The selectivity of the sensor is extremely important for detecting the analyte accurately. To demonstrate the sensing selectivity of single-layer MnO_2 nanosheets toward GSH, several bio-active molecule with reducing ability, such as glucose, fructose, H_2O_2 , uric acid, cysteine (Cys) and 1,4-dithiothreitol (DTT) were used to check whether their presence interfere with the GSH determination. Fig. 5 shows the influence of these reducing bioagents on the absorbance of MnO_2 solution. Results indicate that the addition of these molecules with reducing ability has a slight interference with the detection of GSH since their presence does not result in a significant variation of absorbance, compared to the case of GSH. Especially, Cys has a structure similar to that of GSH, and high concentrations of Cys can also cause MnO_2 reduction. It seems difficult to differentiate GSH from Cys in the detection. Considering that the concentration of Cys (micromolar level) is remarkably lower than that of GSH (millimolar level) in biological systems [29,30,47], this method could be applied for selective detection of GSH.

In addition, several common metal ions in biological fluids, such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cu^+ and Fe^{2+} were added to examine their influence on sensing properties. the absorbance of MnO_2 solution was almost unaffected by the extra addition of a certain amount of Na^+ , K^+ , Mg^{2+} and Ca^{2+} (Fig. 5). Even if several biologically relevant redox active metal ions, such as Cu^+ and Fe^{2+} , were introduced into the colloidal MnO_2 solutions, the absorbance shows a slight response, compared with the case of added GSH. The above results indicate that the chemical response of our present MnO_2 nanosheets toward GSH is selective.

Insert Fig. 5

4. Conclusions

In this paper, single-layer MnO_2 nanosheets were synthesized via a redox reaction between KMnO_4 and sodium dodecyl sulfate by a simple template-free method. The addition of GSH to the synthesized MnO_2 solution at an acidic pH condition leads to the change of solution color depth due to the transition of MnO_2 to Mn(II) , which enables the colorimetric determination of GSH. At a pH of 3.6, The MnO_2 solution responds rapidly toward GSH in a wide analyte concentration range. The sensing is sensitive toward GSH with a detection limit of $0.5\ \mu\text{M}$. The relative standard deviation for seven replicate determinations of GSH was found to be lower than 5.6%. Furthermore, the present sensing material for GSH determination shows a good selectivity. This single-layer MnO_2 -based sensing material might be potential for detecting GSH in biological applications.

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Fig. 1. XRD pattern (a), TEM image (b), FT-IR spectrum (c), XPS spectra of Mn_{2p} (d) and O_{1s} (e), and UV-vis absorption spectrum (f) of the synthesized MnO_2 nanosheets.

Fig. 2. The absorbance of MnO_2 solution after reaction with a given concentration of GSH as a function of pH values. The inset shows the comparison of the MnO_2 solution absorbance with and without the addition of GSH under various pH conditions.

Fig. 3. The absorbance of MnO_2 solution recorded at each time interval of 30 s upon the addition of 50 μL of GSH solution (0.1 mM) to 5 mL of MnO_2 solution (0.05 mM).

Fig. 4. (a) The UV-vis absorption spectra of the MnO_2 solution upon the addition of various concentrations of GSH. (b) The corresponding photographs of the reaction solutions with the addition of different concentrations of GSH ranging from 0 to 0.1 mM (from left to right). (c) The absorbance monitored at 375 nm as a function of GSH concentration.

Fig. 5. The absorbance variations of MnO₂ solution (50μM) upon the addition of various antioxidants and bio-active metal ions. Na⁺, K⁺, Mg²⁺ and Ca²⁺, 100 μM; Cu⁺, Fe²⁺, 50 μM; Glucose, Fructose and uric acid, 100 μM; H₂O₂ and GSH, 50 μM; DTT and Cys, 10 μM.