A Novel Electrochemical Sensor Based on MnO₂/Sepiolite Nanocomposite for the Detection of Hydrogen Peroxide in Human Serum Samples

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Abstract

Background: The reliable and easy-to-operate detection of hydrogen peroxide (H₂O₂) has attracted extensive attention in the fields of biomedicine, food security, and environmental analysis.

Objectives: In this work, a novel electrochemical method was proposed for H₂O₂ monitoring using a carbon paste electrode (CPE) modified with MnO₂/sepiolite nanocomposite.

Methods: MnO₂/sepiolite material was characterized by transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDS), and X-ray diffraction (XRD) technique. The modified CPE was employed for the amperometric monitoring of H₂O₂ in human serum samples.

Results: Electrochemical data showed that the MnO₂/sepiolite-CPE displays a high peak current towards H₂O₂ oxidation. A linear range from 5 to 700 µM and a low detection limit of 0.8 µM for H₂O₂ were obtained with the proposed sensor. Besides, the electrode depicted excellent reproducibility and anti-interferant ability, promising the applicability of this electrochemical method in practical analyses.

Conclusions: This work introduced a new and effective enzyme-less H₂O₂ sensor based on the MnO₂/sepiolite nanocomposite modified CPE. The suggested sensor showed good sensitivity for the rapid detection of H₂O₂ in a wide linear range with a low detection limit and satisfactory reproducibility, which made it practical for the analysis of hydrogen H₂O₂ in real samples.

Keywords: Nanocomposite, Hydrogen peroxide, Sensor, Human Serum

1. Background

H₂O₂ plays an essential mediator in several biological reactions catalyzed by enzymes (1, 2). The excess of H₂O₂ may potentially damage carbohydrates, lipids, and proteins in the human body (3). Thus, it is crucial to design an efficient platform for H₂O₂ measurement in biological samples. So far, different determination schemes have been used for H₂O₂ monitoring, such as chromatography (4), spectrophotometry (5), chemiluminescence (6), and electrochemistry (7).

Enzyme-less H₂O₂ electrochemical sensors have the advantage of simplicity, inexpensive, high sensitivity, rapid response and suitability for real-time detection (8, 9). From this point of view, the construction of new and effective electrochemical assays for H₂O₂ detection, especially in biological samples, has received extensive attention in recent years (10, 11).

2. Objectives

In this work, MnO₂ nanoflakes were deposited on the surface fibrous structure of sepiolite clay via a facile hydrothermal process. The prepared nanocomposite (MnO₂/sepiolite) was employed for the modification of a simple and low-cost carbon paste electrode (CPE). The electrocatalytic activity of the modified CPE toward H₂O₂ was explored. The linear detection range and detection limit of the MnO₂/sepiolite-CPE were also investigated in detail. Furthermore, the fabricated non-enzymatic H₂O₂ electrochemical sensor was used for the determination of H₂O₂ in human serum samples.
3. Methods

3.1. Reagents and Instrument

Flake graphite (100 mesh, 99.5% purity), paraffin oil, H₂O₂ (30 wt%), ammonium persulfate ((NH₄)₂S₂O₈, 99.0 purity), potassium permanganate (KMnO₄, 99.5 purity), disodium hydrogen phosphate dodecahydrate (Na₂HPO₄·12H₂O, 99 purity), and sodium dihydrogen phosphate dehydrate (NaH₂PO₄·2H₂O, 99 purity) were acquired from Merck Co. (Darmstadt, Germany). Sepiolite powder was provided by Dorkav Minig Co., Ltd. (Mashhad, Iran). Raw sepiolite was purified according to a previously reported method (12). Ultrapure water was used for the preparation of phosphate buffer solution.

Electrochemical experiments were conducted on an OriginStat100 electrochemical workstation (OrigaLys, France) using a standard electrochemical cell, including the modified CPE as the working electrode, the platinum wire as the counter electrode, and saturated calomel electrode (SCE) as the reference electrode.

3.2. Synthesis of MnO₂/Sepiolite Nanocomposite

MnO₂/sepiolite nanocomposite was prepared via the one-step hydrothermal method, described in an earlier report (13). Briefly, 2.0 g of the purified sepiolite powder was dispersed into a 30-mL mixed solution of (NH₄)₂S₂O₈ (2.21 g) and KMnO₄ (1.85 g). This mixture was poured into a 50-mL Teflon-lined stainless steel autoclave and then kept in an oven at 110°C for 14 h. The achieved MnO₂/sepiolite material was collected and then washed repeatedly with ultrapure water. The product was further dried in an oven at 60°C.

3.3. Electrode Fabrication

The working CPE electrode was prepared according to the methods reported previously (14, 15). Typically, materials, including graphite, paraffin oil, and MnO₂/sepiolite in the ratio of 67:25:8 (w/w), were mixed in a mortar for 10 min to get a homogenized carbon paste. The obtained paste was filled carefully into a Teflon tube (3 mm inner diameter and a height of 10 cm) as the body of the electrode. A copper wire was used as the electrical conductor. A fresh CPE surface was provided with polishing the electrode surface on a weighing paper.

4. Results

X-ray diffraction patterns of the sepiolite and MnO₂/sepiolite samples are shown in Figure 1A and B. The diffraction peaks appeared at 2θ = 7.7°, 19.6°, 20.7°, 26.5°, and 34.8° matched well with the diffraction peaks of (110), (060), (131), (080), and (441) crystal planes of sepiolite clay standard data (JCPDS card PDF file No. 13-0595) (16, 17). Two characteristic diffraction peaks at 37.2° and 66.3° could also be assigned to the (131), and (421) planes of γ-MnO₂ (JCPDS 72-1982), respectively (18).

Figure 1. X-ray diffraction patterns of A, sepiolite; and B, MnO₂/sepiolite nanocomposite.

The TEM images of the natural sepiolite and MnO₂/sepiolite nanocomposite are shown in Figure 2A and B. As can be seen, MnO₂ nanoflakes successfully deposited on the surface of the sepiolite fibers. Moreover, the EDS spectrum of the MnO₂/sepiolite nanocomposite (Figure 3) depicts the existence of Mn, O, Si, and Mg and elements in the prepared material. All the above results confirm the synthesis of MnO₂/sepiolite nanocomposite via the hydrothermal method.

5. Discussion

The electrochemical performances of unmodified CPE and MnO₂/sepiolite-CPE toward H₂O₂ were studied by cyclic voltammetry. As presented in Figure 4, the oxidation peak current for MnO₂/sepiolite-CPE (appeared at 0.45 V) was much larger than that of the unmodified CPE, which is ascribed to the remarkable catalytic ability of MnO₂/sepiolite material toward H₂O₂ oxidation on the electrode surface.

The influence of solution pH was explored on the voltammetric peak current at the MnO₂/sepiolite-CPE. As

seen in Figure 5A, the voltammetric signals first increased with increasing pH up to 7.0, and then decreased at higher pH values. Thus, the pH 7.0 of phosphate buffer was selected for the following electrochemical tests.

The effect of MnO$_2$/sepiolite dose on the range of 4.0 - 12.0% (w/w) was studied by the voltammetric method in a solution containing 100 $\mu$M of H$_2$O$_2$. As shown in Figure 5B, the maximum response can be observed at the amount of 8.0% MnO$_2$/sepiolite. Consequently, it was chosen as the optimal modifier amount in the next experiments.

To assess the sensitive response towards H$_2$O$_2$, the current-time (I-t) curve was explored at an applied potential of 0.5 V. The amperometric responses of the MnO$_2$/sepiolite-CPE with the successive injection of H$_2$O$_2$ into 0.1-M buffer solution (pH 7.0) were investigated, and the results are depicted in Figure 6. The linear relationship between amperometric signal current and analyte concentration in the range of 5 - 700 $\mu$M could be observed. Furthermore, the limit of detection (based on 3$\sigma$) was found to be 0.8 $\mu$M, which was less than that of other methods (19-24) as listed in Table 1. Besides, the relative standard deviation (RSD) for ten replicate detections of 50 $\mu$M H$_2$O$_2$ was calculated as 2.6%. It was also noticed that the MnO$_2$/sepiolite-CPE showed good stability and could be used for at least two weeks. The influence of common interfering species on the determination of 50 $\mu$M H$_2$O$_2$ using the MnO$_2$/sepiolite-CPE was evaluated. As listed in Table 2, the 10-fold concentration of interfering molecules demonstrated nearly no interference in H$_2$O$_2$ monitoring. This finding indicated the satisfactory selectivity of the suggested assay.

The practical applications of the MnO$_2$/sepiolite-CPE in analysis of H$_2$O$_2$ in human serum samples were studied using the standard addition method. Real samples were provided from a local hospital in Tehran. The obtained results and recoveries of the spiked samples are exhibited in Table 3. These results showed that the present system is an effective platform for the monitoring of H$_2$O$_2$ in real applications.
**Figure 5.** The effect of A, pH; and B, MnO$_2$/sepiolite amount on voltammetric current response at MnO$_2$/sepiolite-CPE in phosphate buffer (0.1 M) containing 100 µM of H$_2$O$_2$.

**Table 1.** Comparative Study of Various Electrochemical Sensors for H$_2$O$_2$ Detection

<table>
<thead>
<tr>
<th>Electrode Modifier*</th>
<th>Linear Range (µM)</th>
<th>Detection Limit (µM)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnO$_2$ nanotubes/reduced graphene oxide nanocomposite</td>
<td>100 - 30000</td>
<td>1.29</td>
<td>(19)</td>
</tr>
<tr>
<td>V$_2$O$_5$/VO$_2$ nanostructures</td>
<td>8 - 215</td>
<td>5</td>
<td>(20)</td>
</tr>
<tr>
<td>Cuprous oxide-reduced graphene oxide nanocomposites</td>
<td>30 - 12800</td>
<td>21.7</td>
<td>(21)</td>
</tr>
<tr>
<td>Poly(p-aminobenzene sulfonic acid)</td>
<td>50 - 550</td>
<td>10</td>
<td>(22)</td>
</tr>
<tr>
<td>Hematite nanoparticles</td>
<td>50 - 3145</td>
<td>22</td>
<td>(23)</td>
</tr>
<tr>
<td>Gold nanobipyramids/multi-walled carbon nanotubes</td>
<td>5.0 - 47300</td>
<td>1.5</td>
<td>(24)</td>
</tr>
<tr>
<td>MnO$_2$/sepiolite nanocomposite</td>
<td>5 - 700</td>
<td>0.8</td>
<td>This work</td>
</tr>
</tbody>
</table>

**Figure 6.** Amperometric current–time curve of MnO$_2$/sepiolite-CPE to consecutive addition a series of concentration (5.0 to 700.0 µM) of H$_2$O$_2$ into 0.1 M buffer solution (pH = 7.0) at an applied potential of 0.5 V (Inset: calibration plot of sensor).

**Table 2.** Effects of Interfering Species on H$_2$O$_2$ Detection

<table>
<thead>
<tr>
<th>Foreign Molecule</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>97.9</td>
</tr>
<tr>
<td>Citric acid</td>
<td>98.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>97.7</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>97.2</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>98.6</td>
</tr>
</tbody>
</table>

5.1. Conclusion

In sum, a simple, selective, and sensitive electrochemical device for H$_2$O$_2$ determination was proposed. The MnO$_2$/sepiolite-CPE showed a good linear relationship with the concentration of H$_2$O$_2$ up to 700 µM. Moreover, the suggested method showed notable selectivity for the measuring of H$_2$O$_2$ in the presence of some interfering species. In addition, MnO$_2$/sepiolite-CPE demonstrated...
great potential application for $\text{H}_2\text{O}_2$ monitoring in real biological samples.

Footnotes

Authors' Contribution: AbduRahman Hosseinifar, collected the electrochemical data; Masoud Ghanei-Motlagh, developed the original idea and the protocol, abstracted and analyzed data, wrote the manuscript, and is a guarantor; Maryam Fayazi, analysis and interpretation of data and material support. All authors read and approved the final manuscript.

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References


