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A Novel Electrochemical Sensor Based on MnO_2/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 (H_2O_2) 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 H_2O_2 monitoring using a carbon paste electrode (CPE) modified with MnO_2/sepiolite nanocomposite.
Methods: MnO_2/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 H_2O_2 in human serum samples.
Results: Electrochemical data showed that the MnO_2/sepiolite-CPE displays a high peak current towards H_2O_2 oxidation. A linear range from 5 to 700 μM and a low detection limit of 0.8 μM for H_2O_2 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 H_2O_2 sensor based on the MnO_2/sepiolite nanocomposite modified CPE. The suggested sensor showed good sensitivity for the rapid detection of H_2O_2 in a wide linear range with a low detection limit and satisfactory reproducibility, which made it practical for the analysis of hydrogen H_2O_2 in real samples.

Keywords: Nanocomposite, Hydrogen peroxide, Sensor, Human Serum

1. Background

H_2O_2 plays an essential mediator in several biological reactions catalyzed by enzymes (1, 2). The excess of H_2O_2 may potentially damage carbohydrates, lipids, and proteins in the human body (3). Thus, it is crucial to design an efficient platform for H_2O_2 measurement in biological samples. So far, different determination schemes have been used for H_2O_2 monitoring, such as chromatography (4), spectrophotometry (5), chemiluminescence (6), and electrochemistry (7).

Enzyme-less H_2O_2 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 H_2O_2 detection, especially in biological samples, has received extensive attention in recent years (10, 11).

2. Objectives

In this work, MnO_2 nanoflakes were deposited on the surface fibrous structure of sepiolite clay via a facile hydrothermal process. The prepared nanocomposite (MnO_2/sepiolite) was employed for the modification of a simple and low-cost carbon paste electrode (CPE). The electrocatalytic activity of the modified CPE toward H_2O_2 was explored. The linear detection range and detection limit of the MnO_2/sepiolite-CPE were also investigated in detail. Furthermore, the fabricated non-enzymatic H_2O_2 electrochemical sensor was used for the determination of H_2O_2 in human serum samples.

3. Methods

3.1. Reagents and Instrument

Flake graphite (100 mesh, 99.5% purity), paraffin oil, H_2O_2 (30 wt%), ammonium persulfate ($(NH_4)_2S_2O_8$, 99.0 purity), potassium permanganate ($KMnO_4$, 99.5 purity), disodium hydrogen phosphate dodecahydrate (Na_2HPO_4 $12H_2O$, 99 purity), and sodium dihydrogen phosphate dehydrate (NaH_2PO_4 $2H_2O$, 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 a OrigaState100 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

MnO_2/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 $(NH_4)_2S_2O_8$ (2.21 g) and KMnO4 (1.85 g). This mixture was poured into a 50-mL Tefon-lined stainless steel autoclave and then kept in an oven at 110°C for 14 h. The achieved MnO_2/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 flake graphite, paraffin oil, and MnO_2/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 MnO_2/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-MnO_2 (JCPDS 72-1982), respectively (18).

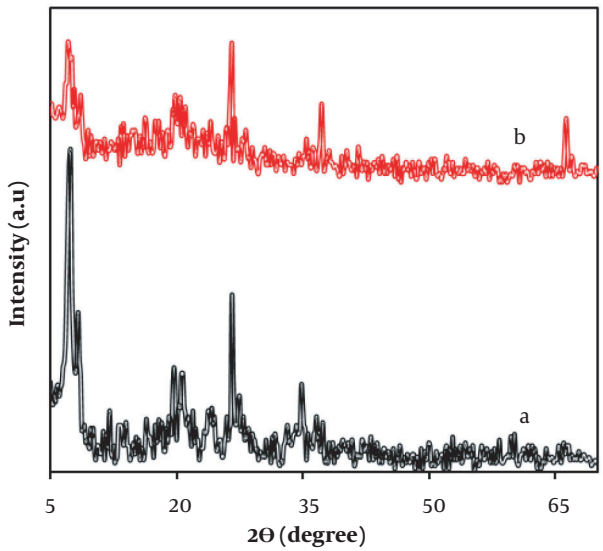

Figure 1. X-ray diffraction patterns of A, sepiolite; and B, MnO_2/sepiolite nanocomposite.

The TEM images of the natural sepiolite and MnO_2/sepiolite nanocomposite are shown in Figure 2A and B. As can be seen, MnO_2 nanoflakes successfully deposited on the surface of the sepiolite fibers. Moreover, the EDS spectrum of the MnO_2/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 MnO_2/sepiolite nanocomposite via the hydrothermal method.

5. Discussion

The electrochemical performances of unmodified CPE and MnO_2/sepiolite-CPE toward H_2O_2 were studied by cyclic voltammetry. As presented in Figure 4, the oxidation peak current for MnO_2/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 MnO_2/sepiolite material toward H_2O_2 oxidation on the electrode surface.

The influence of solution pH was explored on the voltammetric peak current at the MnO_2/sepiolite-CPE. As

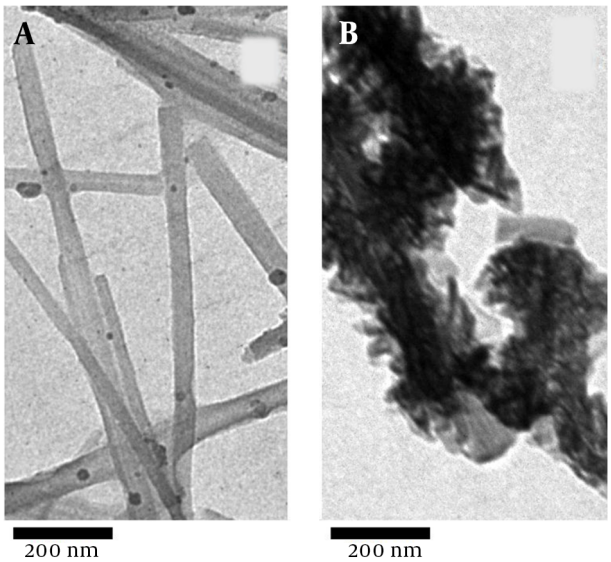

Figure 2. SEM images A, sepiolite; and B, MnO_2/sepiolite nanocomposite.

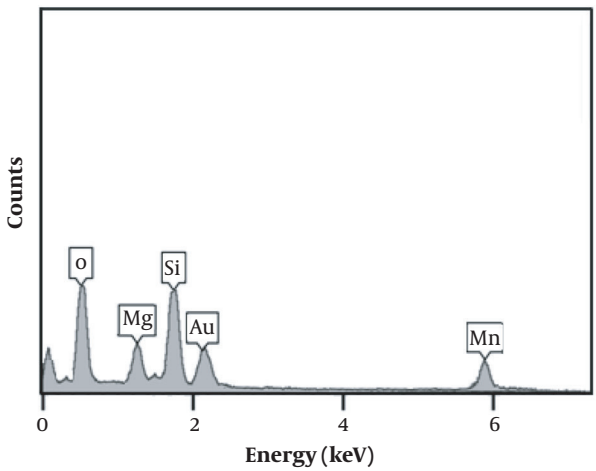

Figure 3. EDS spectrum of MnO_2/sepiolite nanocomposite

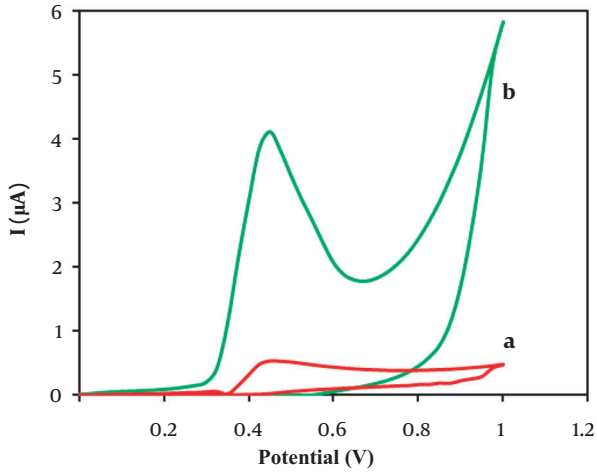

Figure 4. Cyclic voltammograms of the unmodified and modified electrodes in 0.1 M pH = 7 phosphate buffer at 100 μM of H_2O_2, scan rate: 50 $mV.s^{-1}$.

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 μM of H_2O_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_2O_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_2O_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 μM could be observed. Furthermore, the limit of detection (based on 3σ) was found to be 0.8 μM, 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 μM H_2O_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 μM H_2O_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_2O_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_2O_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_2O_2 in real applications.

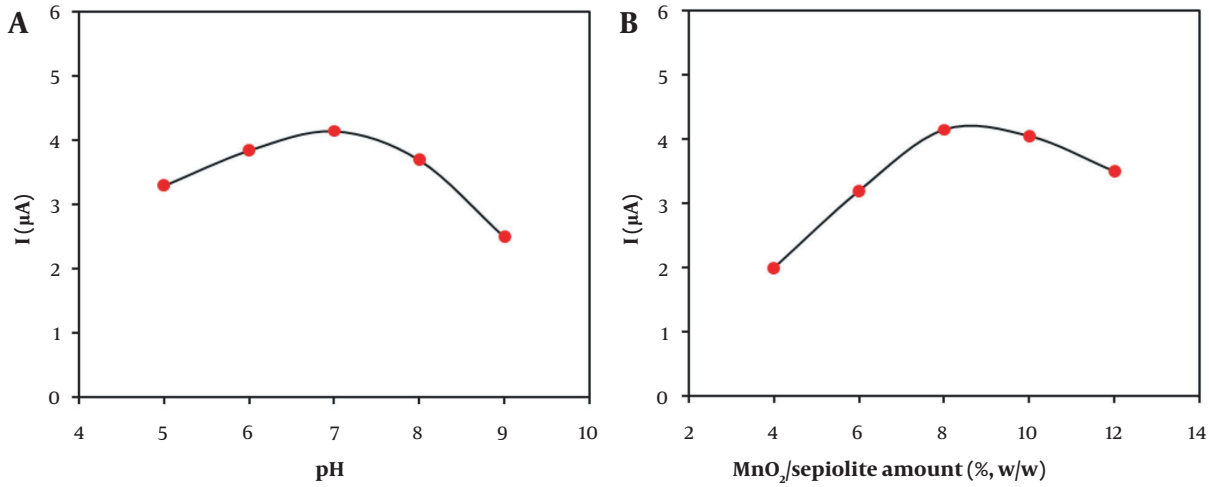

Figure 5. The effect of A, pH; and B, MnO_2/sepiolite amount on voltammetric current response at MnO_2/sepiolite-CPE in phosphate buffer (0.1 M) containing 100 μM of H_2O_2.

Table 1. Comparative Study of Various Electrochemical Sensors for H_2O_2 Detection

Electrode Modifier*	Linear Range (μM)	Detection Limit (μM)	Ref.
MnO_2 nanotubes/reduced graphene oxide nanocomposite	100 - 30000	1.29	(19)
V_2O_5/VO_2 nanostructures	8 - 215	5	(20)
Cuprous oxide-reduced graphene oxide nanocomposites	30 - 12800	21.7	(21)
Poly(p-aminobenzene sulfonic acid)	50 - 550	10	(22)
Hematite nanoparticles	50 - 3145	22	(23)
Gold nanobipyramids/multi-walled carbon nanotubes	5.0 - 47300	1.5	(24)
MnO_2/sepiolite nanocomposite	5 - 700	0.8	This work

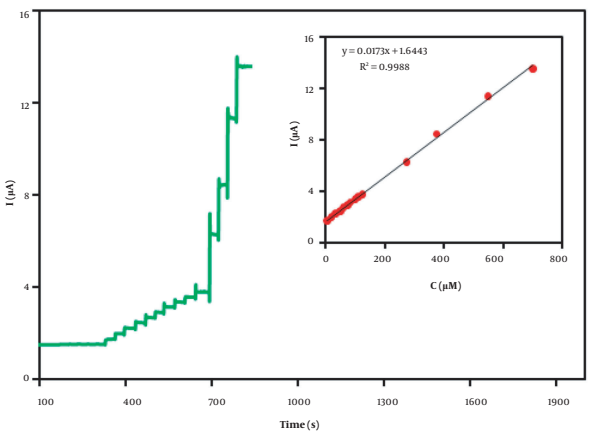

Figure 6. Amperometric current-time curve of MnO_2/sepiolite-CPE to consecutive addition a series of concentration (5.0 to 700.0 μM) of H_2O_2 into 0.1 M buffer solution (pH = 7.0) at an applied potential of 0.5 V (Inset: calibration plot of sensor).

Table 2. Effects of Interfering Species on H_2O_2 Detection

Foreign Molecule	Recovery (%)
Sucrose	97.9
Citric acid	98.0
Glucose	97.7
Ascorbic acid	97.2
Glutamic acid	98.6

5.1. Conclusion

In sum, a simple, selective, and sensitive electrochemical device for H_2O_2 determination was proposed. The MnO_2/sepiolite-CPE showed a good linear relationship with the concentration of H_2O_2 up to 700 μM. Moreover, the suggested method showed notable selectivity for the measuring of H_2O_2 in the presence of some interfering species. In addition, MnO_2/sepiolite-CPE demonstrated

Table 3. H_2O_2 Detection in Human Serum Samples

Sample	Spiked (μM)	Found (μM)	Recovery (%)	RSD [a]
Human serum (1)	10	9.8	98.0	3.5
	20	20.3	101.5	4.1
	30	29.4	102.0	3.4
Human serum (2)	10	10.3	103.0	3.5
	20	19.7	98.5	4.2
	30	28.9	96.3	4.0

[a] n = 3.

great potential application for H_2O_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.

Conflict of Interests: The authors report no conflicts of interest in this work.

Data Reproducibility: The data presented in this study are openly available in one of the repositories or will be available on request from the corresponding author by this journal representative at any time during submission or after publication. Otherwise, all the consequences of possible withdrawal or future retraction will be with the corresponding author.

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