

Full Paper

A highly Sensitive Electrochemical Determination of Norepinephrine at a Graphite Screen Printed Electrode Modified with Mn₃O₄ Nanoparticles

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Abstract- A novel electrochemical sensor consisting of Mn₃O₄ nanoparticles modified graphite screen printed electrode (Mn₃O₄ NPs/SPE) was fabricated and applied for the determination of norepinephrine (NEP). Electroanalytical measurements including cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used. The optimum buffer of pH 7.0, the norepinephrine oxidation peak current enhanced linearly with concentration ranging from 0.7 to 400.0 μM (R² of 0.9994) with a detection limit of 0.1 μM. The proposed sensor was used for determination of norepinephrine in real samples.

Keywords- Norepinephrine, Mn₃O₄ nanoparticles, Graphite screen printed electrode, Voltammetry

1. INTRODUCTION

Norepinephrine (NEP) is a catecholamine neurotransmitter in the brain, and it has the important physiological effects as a neurotransmitter and hormone in the circulatory system. Consequently, determination of its content in human blood or urine can be used to diagnose some diseases [1,2], which is of vital significance. At present, various methods have been established to determine the concentration of norepinephrine in human blood or urine,

including chromatography (HPLC), capillary electrophoresis (CE), thin-layer chromatography (TLC), spectrophotometry, fluorescence spectrometry and electrochemical biosensors [3-9]. Although some of the methods have high sensitivity and specificity, complicated and expensive instruments are required. Consequently, electrochemical sensors are becoming important tools in medical, biological and environmental analysis due to their simplicity, high sensitivity and relative cheapness [10-20].

Screen-printed carbon electrodes (SPEs) show a widely accessible, disposable electrochemical sensor, inexpensive, simple and non-toxic [21]. Replacement of conventional electrochemical cells by SPEs connected to miniaturized potentiostats is the main trend in the shift of lab electrochemical equipment to hand-held field analyzers [22]. They are also suitable for working with microvolumes and for decentralized assays (point of care tests), etc [23,24]. Furthermore, the sensitivity and specificity of these electrochemical sensors, can be considerably improved by the design and development of new materials as a modifier [25,26].

The application of nanomaterials to modified sensors enhances the sensitivity of recent developments in materials chemistry research are accompanied by the synthesis of new structured metal oxide materials with novel properties [27,28]. These metal oxide materials offer excellent electronic, conductivity and catalytic properties, and electrocatalytic activity, which accelerate electron transfer between the electrode surface and redox species [29-32]. These unique structured metal oxide materials have been applied to electroanalysis of drugs in various preparation methods [33]. The design and synthesis of unique structured metal oxide on electrode surfaces were performed to improve the sensitivity of electrochemical measurements by increasing the surface area or using the catalytic activity of metal oxide materials and the transition metal oxides have been used for the anticancer activities [34]. Among the transition metal oxides Mn_3O_4 materials have been also utilized as a drug in the treatment of cancer [35]. Mn_3O_4 (hausmannite) have an outstanding theoretical capacity of electrochemical activity, low-cost and have high surface area; as a result, it has been extensively employed as an electrode material in batteries and supercapacitor [36-39]. It's an important metal oxide that is widely used as an active catalyst for the oxidation of many materials [40-42]. Owing to the various versatile characteristic features, including large surface area and the folded architecture of nanoparticles had promoted it as an attractive material for electrochemical sensor and biosensor applications.

According to the previous points, it is important to create suitable conditions for detection of norepinephrine in biological fluids. In this study, we describe application of novel Mn_3O_4 nanoparticles as a nanostructure sensor for voltammetric determination of norepinephrine. The proposed sensor showed good electrocatalytic effect on norepinephrine. The modified electrode shows advantages in terms of selectivity, reproducibility and sensitivity.

Eventually, we evaluate the analytical performance of the suggestion sensor for norepinephrine determination in real sample.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. The screen-printed electrode (DropSens, DRP-110, Spain) consists of three main parts which are a graphite counter electrode, a silver pseudo-reference electrode and a graphite working electrode.

All solutions were freshly prepared with double distilled water. Norepinephrine and all other reagents were of analytical grade and were obtained from Merck chemical company (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-9.0.

2.2. Construction of modified electrode

The bare graphite screen printed electrode was coated with Mn_3O_4 nanoparticles as follows. A stock solution of Mn_3O_4 nanoparticles in 1 ml aqueous solution was prepared by dispersing 1 mg Mn_3O_4 nanoparticles with ultrasonication for 1 h, and a 5 μl aliquot of the Mn_3O_4 nanoparticles/ H_2O suspension solution was casted on the carbon working electrodes, and waiting until the solvent was evaporated in room temperature.

3. RESULTS AND DISCUSSION

3.1. Electrocatalytic oxidation of norepinephrine at a Mn_3O_4 NPs/SPE

The electrochemical behavior of norepinephrine is dependent on the pH value of the aqueous solution. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of norepinephrine. Thus the electrochemical behavior of norepinephrine was studied in 0.1 M PBS in different pH values ($2.0 < \text{pH} < 9.0$) at the surface of Mn_3O_4 NPs/SPE by CV. It was found that the electrocatalytic oxidation of norepinephrine at the surface of Mn_3O_4 NPs/SPE was more favored under neutral conditions than in acidic or basic medium. Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of norepinephrine oxidation at the surface of Mn_3O_4 NPs/SPE.

Fig. 1 depict the cyclic voltammetric responses for the electrochemical oxidation of 400.0 μM norepinephrine at Mn_3O_4 NPs/SPE (curve a) and bare SPE (curve b). The anodic peak potential for the oxidation of norepinephrine at Mn_3O_4 NPs/SPE (curve a) is about 200 mV

compared with 245 mV for that on the bare SPE (curve b). Similarly, when the oxidation of norepinephrine at the Mn_3O_4 NPs/SPE (curve a) and bare SPE (curve b) are compared, an extensive enhancement of the anodic peak current at Mn_3O_4 NPs/SPE relative to the value obtained at the bare SPE (curve b) is observed. In other words, the results clearly indicate that the Mn_3O_4 nanoparticles improve the norepinephrine oxidation signal.

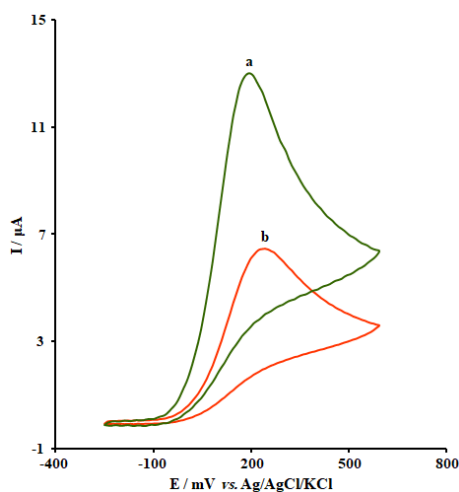


Fig. 1. Cyclic voltammograms of (a) Mn_3O_4 NPs/SPE and (b) bare SPE in 0.1 M PBS (pH 7.0) in the presence of 400.0 μM norepinephrine at the scan rate 50 mVs^{-1}

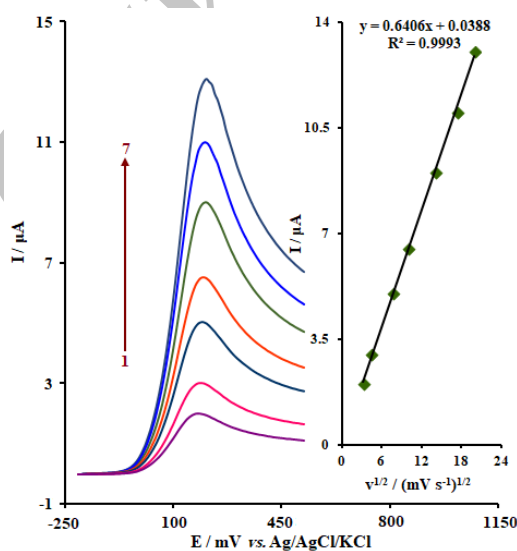
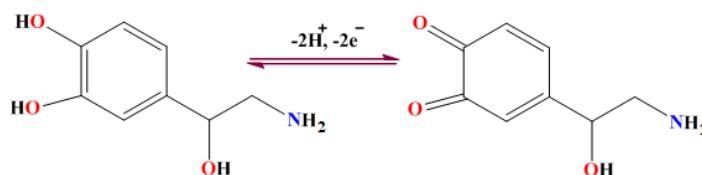


Fig. 2. Cyclic voltammograms of Mn_3O_4 NPs/SPE in 0.1 M PBS (pH 7.0) containing 200.0 μM norepinephrine at various scan rates; numbers 1-7 correspond to 10, 20, 60, 100, 200, 300 and 400 mV s^{-1} , respectively. Inset: Variation of cathodic peak current vs. $v^{1/2}$

The effect of potential scan rates on the oxidation current of norepinephrine has been studied (Fig. 2). The results showed that increasing in the potential scan rate induced an increase in the peak current. In addition, the oxidation process is diffusion controlled as deduced from the linear dependence of the anodic peak current (I_p) on the square root of the potential scan rate ($v^{1/2}$) over a wide range from 10 to 400 mV s^{-1} (Fig. 2). The total electrochemical reaction of norepinephrine at Mn_3O_4 NPs/SPE is shown in scheme 1.



Scheme 1. Electrochemical oxidation mechanism of norepinephrine at modified electrode

3.2. Chronoamperometric measurements

Chronoamperometric measurements of norepinephrine at Mn_3O_4 NPs/SPE were carried out by setting the working electrode potential at 0.3 V for the various concentration of norepinephrine in PBS (pH 7.0) (Fig. 3).

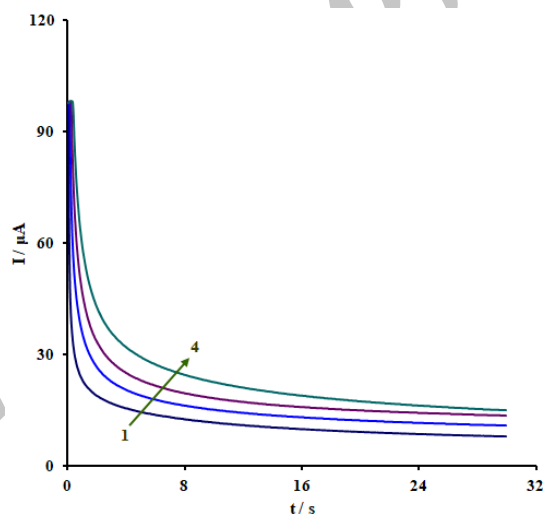


Fig. 3. Chronoamperograms obtained at Mn_3O_4 NPs/SPE in 0.1 M PBS (pH 7.0) for different concentration of norepinephrine. The numbers 1–4 correspond to 0.2, 1.1, 2.0 and 2.5 mM of norepinephrine

For an electroactive material (norepinephrine in this case) with a diffusion coefficient of D , the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [43].

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2}$$

Where D and C_b are the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and the bulk concentration (mol cm^{-3}), respectively. Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of norepinephrine (Fig. 4A). The slopes of the resulting straight lines were then plotted vs. norepinephrine concentration (Fig. 4B). From the resulting slope and Cottrell equation the mean value of the D was found to be $2.3 \times 10^{-5} \text{ cm}^2/\text{s}$.

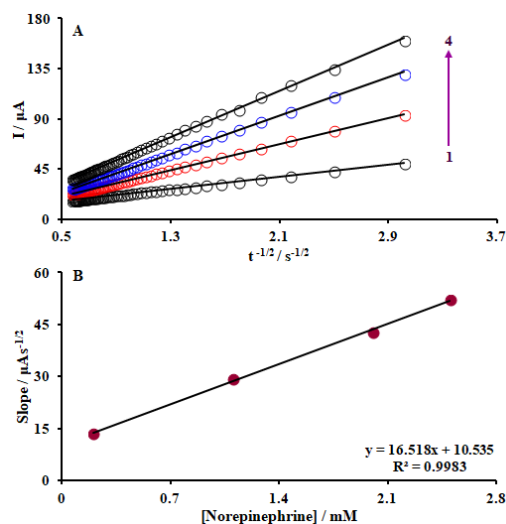


Fig. 4. (A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 1–4; (B) Plot of the slope of the straight lines against norepinephrine concentration

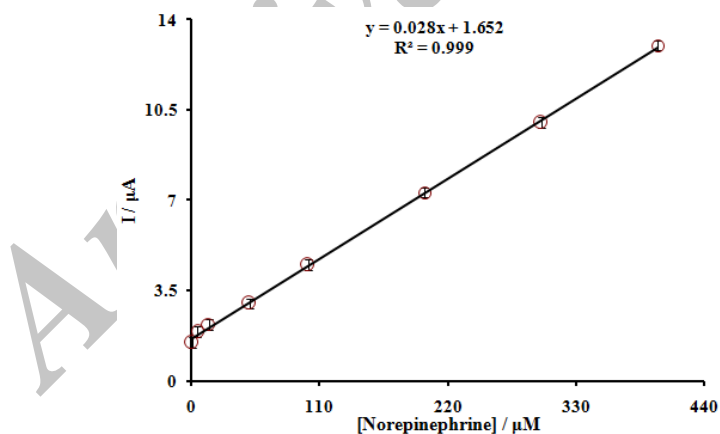


Fig. 5. Plot of the electrocatalytic peak current as a function of norepinephrine concentration in the range of 0.7, 5.0, 15.0, 50.0, 100.0, 200.0, 300.0 and 400.0 μM

3.3. Calibration plot and limit of detection

The peak current of norepinephrine oxidation at the surface of the modified electrode can be used for determination of norepinephrine in solution. Therefore, differential pulse

voltammetry (DPV) experiments were done for different concentrations of norepinephrine. The oxidation peak currents of norepinephrine at the surface of a modified electrode were proportional to the concentration of the norepinephrine within the ranges 0.7 to 400.0 μM (Fig. 5). The detection limit (3σ) of norepinephrine was found to be 1.0×10^{-7} M. These values are comparable with values reported by other research groups for the determination of norepinephrine at the surface of modified electrodes (see Table 1).

Table 1. Comparison of the efficiency of some methods used in detection of norepinephrine.

Modified electrode	Detection samples	LDR	Ref.
Eriochrome Cyanine R GCE	PBS (pH 7.0)	2.0-50.0 μM	[44]
DDP-CNTs CPE	Norepinephrine injection	2.0-30.0 μM	[45]
CNTs CPE	Norepinephrine injection	15.0-25.0 μM	[46]
Carbon-coated nickel GCE	PBS (pH 7.0)	0.2-80.0 μM	[47]
ZrO ₂ -CNTs CPE	Norepinephrine injection	5.0-20.0 μM	[48]
MWNTs-ZnO/chitosan SPE	Cerebrospinal fluid	1.0-30.0 μM	[49]
Mn ₃ O ₄ nanoparticles SPE	PBS (pH 7.0)	0.7-400.0 μM	This work

3.4. Analysis of real samples

To assess the applicability of the application of the modified electrode for the determination of norepinephrine in real samples, the described method was applied to the determination of norepinephrine in norepinephrine ampoule and urine samples. For the purpose of this analysis the standard addition method was used and the results are given in Table 2.

Table 2. The application of Mn₃O₄ NPs/SPE for determination of norepinephrine in norepinephrine ampoule and urine samples (n=5). All concentrations are in μM

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Urine	0	5.0	-	2.7
	2.5	7.4	98.7	2.9
	5.0	10.3	103.0	3.5
	7.5	12.5	99.2	2.1
	10.0	15.2	101.3	1.9
	0	-	-	-
	5.0	5.1	102.0	3.4
	10.0	9.9	99.0	2.7
	15.0	14.6	97.3	2.8
	20.0	20.2	101.0	1.7

The observed recovery of norepinephrine was satisfactory and the reproducibility of the results was demonstrated based on the mean relative standard deviation (R.S.D.).

4. CONCLUSION

This work demonstrates the construction of a modified screen printed electrode by the incorporation of Mn_3O_4 nanoparticles. The electrochemical behavior of the norepinephrine was studied by cyclic voltammetry. The results showed that the peak potential of norepinephrine shifted by 45 mV to a less positive potential at the surface of the modified electrode. A low detection limit, together with the ease of preparation and regeneration of the electrode surface, as well as a long time of stability and reproducibility, makes the system discussed above useful in the construction of simple devices for the determination of norepinephrine. Finally the modified electrode was used for determination of norepinephrine in norepinephrine ampoule and urine samples.

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