



A novel electrochemical sensor for determination of morphine in a sub-microliter of human urine sample

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ABSTRACT

A novel electrochemical sensor was fabricated for measurement the concentration of morphine in very low volume of solution. This paper introduces a new approach for sensing of morphine (MP) in sub microliter of the solution using a modified carbon paste electrode. The electrode was prepared simply by mixing a hydrogel as an absorbent polymeric matrix with a carbon paste. Unlike the conventional electrochemical methods, a drop of morphine solution was absorbed into the surface of the electrode, and then the electrode was immersed into an electrochemical cell. The electrode showed a dynamic concentration range from 5.0 to 200 μM with a detection limit of 1 μM at pH 7 with respect to the concentration of morphine. The electrode successfully applied for determination of morphine in a drop of urine sample.

1. Introduction

There is a great need for the use of analytical techniques that consume low volumes of analyte solutions. The determination of analytes in small volume of solutions is critical, especially in biological and rare samples such as DNA, RNA, and living cells [1]. Such low volume or droplet-based techniques have a low sample loss (or low waste) and also lower costs compared to the conventional analytical techniques. Droplet-based systems are valuable tools for various applications, such as single-cell analysis [2,3], diagnostics [4], DNA sequencing [5] and drug screening [6].

Morphine (MP) is a phenolic compound and an alkaloid which can cause disruption in the central nervous system. It is frequently used to alleviate severe pains in patients, especially after a surgical operation. MP is toxic in high dose and it can be fatal. To prevent overdose, it is necessary for clinical medicine to sensitively determine the concentrations of morphine in a patient's urine or blood [7]. Since, MP is presented in the urine sample of patients and addicts at trace levels, a sensitive analytical method is desirable for its quantitative measurement. Various analytical methods such as chromatography [8], capillary electrophoresis [9], spectrometry [10], immunoassay [11] and some electrochemical methods [12–20] have been developed for determination of morphine in sample solutions. Among these instrumental methods, the electrochemical techniques have more importance because of good sensitivity, low cost and fast operation. Morphine is an electroactive compound. According to the literatures [14], the first oxidation of morphine is related to the oxidation of the phenolic group

and the second oxidation is related to the oxidation of the tertiary amine group of morphine.

In this research work, a simple modified carbon paste electrode was used for determination of morphine in solutions. The bare carbon paste electrode (CPE) had poor sensitivity towards the determination of morphine. In order to improve the electrochemical characteristic of the electrode, a modification process was employed with a hydrogel. The hydrogel is a three-dimensional cross-linked hydrophilic polymer capable of swelling and de-swelling in water. These kind of polymers, can absorb and retain fluids such as water, blood and urine. The ability of hydrogels to absorb liquids arises from the porosity and the presence of hydrophilic functional groups such as NH_2 , COOH , OH , CONH_2 and etc. in their structure [21–25].

In the present work, for a first time a novel droplet-based sensor was constructed for determination of morphine in sub microliter of solution. By mixing the hydrogel with the carbon paste, a hydrogel modified carbon paste electrode (HCPE) was constructed. Unlike the conventional electrochemical methods, a sub microliter of the morphine solution was inserted directly into the surface of the electrode and then the electrode containing the morphine was immersed into an electrochemical cell.

2. Experimental

2.1. Apparatus

The electrochemical experiments were performed in 4 mL of a

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phosphate buffer solution (0.1 M) at pH 7.0. The cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed using a μ -Autolab electrochemical system (Eco-Chemie, The Netherlands, NOVA software) coupled with a conventional three-electrode cell. A hydrogel-carbon paste electrode (HCPE) was used as a working electrode and a platinum wire and Ag/AgCl electrodes (both from Azar electrode Co, Urmia, Iran) were used as auxiliary and reference electrodes, respectively. The cyclic voltammograms were recorded from 0.1 V to 1 V at scan rate of 100 mV/s. Scanning electron micrographs were obtained using a scanning electron microscope (SEM, LEO 1450VP, Germany). Also a pH meter (Sana SL.901, Iran) was used for measurement of the pH of the solutions.

2.2. Chemicals

All chemicals were of analytical-reagent grade. Chitosan with molecular weight of 100,000–300,000 was purchased from EXIR Company (Österreich). Morphine hydrochloride (MP) was prepared from Temad Company (Iran) and a stock solution (1 mM) of it was prepared in a phosphate buffer solution (PBS, 0.1 M) and stored at refrigerator in a dark place. The standard solutions were prepared by diluting the stock solution with adequate buffer solution. The hydrogel was synthesized as described in our previous work [21]. It is made of acrylamide and acrylic acid as monomers and *N,N'*-methylenebisacrylamide as a cross-linker. The synthesized hydrogel is a pH responsive gel because of containing both acidic (COOH) and basic groups (NH₂) in its network.

2.3. Preparation of the hydrogel modified carbon paste electrode

The hydrogel modified carbon paste was prepared by mixing the different amounts of the hydrogel with graphite powder (totally 700 mg) and 100 μ L of paraffin oil. The homogeneous paste was packed into a 1 mL insulin plastic syringe and its electrical contact was obtained via a copper wire. The prepared electrode was abbreviated as HCPE. Prior to use, the surface of each electrode was polished using a weighing paper. Also the surface of the electrode was renewed simply by pushing the paste with the copper wire and further polishing process. The prepared electrodes were stored at refrigerator. For determination of morphine, sub microliter of morphine solution was inserted into the surface of the electrode. Since the hydrogel can absorb water from the solution, immediately a sub microliter of chitosan solution was also dropped at the surface of the electrode. The chitosan acts as a covering film and prevents the hydrogel from further absorption of water and also prevents from morphine leakage into the electrolyte solution. After drying the electrode at room temperature (10 min), the dried electrode was dipped into an electrochemical cell.

3. Results and discussion

3.1. Electrode characterization

The morphological characteristics of the bare HCPE (without chitosan) and HCPE modified with chitosan were analyzed by scanning electron microscopy (SEM). Fig. 1, shows the SEM image of the two electrodes. The surface morphology of the electrodes confirms that the chitosan, as a surface modifying agent, exactly covers the surface of the electrode homogeneously (Fig. 1B).

3.2. Investigation of the electrochemical behavior of morphine at the surface of the modified electrode

The electrochemical behavior of morphine at different modified carbon paste electrodes was investigated by cyclic voltammetry between the potential of 0.1 and 1 V with scan rate of 100 mV/s in PBS (pH 7). Fig. 2, shows the typical CVs of morphine (150 μ M) at the different modified electrodes. An anodic peak is appeared for the

morphine molecules at 0.76 V which is improved by increasing the amount of hydrogel from 0.05 to 0.125 g. Therefore, the best hydrogel carbon paste electrode was prepared by mixing 0.125 g hydrogel, 0.575 g graphite powder and 100 μ L of paraffin oil. At higher amount of the hydrogel, the conductivity of the paste was decreased and thereby, the intensity of the anodic peak current was reduced.

According to the literatures [7], the irreversible anodic peak which is appeared at 0.76 V, is attributed to the oxidation of the phenolic group of morphine molecules which leads to the formation of pseudo-morphine molecules.

3.3. The effect of pH

The effect of pH on the electrochemical response of morphine was also studied. For this purpose, a series of phosphate buffer solution (0.1 M) in the pH range of 5.0 to 10.0 was prepared. Fig. 3, shows the effect of pH on the anodic peak current of morphine. As is evident in this Figure, the anodic peak potential of morphine (E_{pa}) shifts a little to the negative direction with increasing the pH and the anodic peak current increases up to 7 and then decreases. Therefore, the next experiments were performed in PBS buffer solutions at pH 7.

3.4. The effect of potential scan rate

The effect of scan rate on the response of morphine at the hydrogel modified carbon paste electrode was also examined by cyclic voltammetry. The CV voltammograms were recorded with a sub microliter of morphine solution with concentration of 150 μ M. The results show that with increasing the scan rate from 10 to 200 mV/s, the anodic peak current increases gradually and also the oxidation peak potential shifts to more positive direction. There is also a linear relationship between the log of oxidation peak current (log I_p) and the log of the scan rate with a linear equation: $\log I_p = 0.663 \log(v) + 0.044$ with R² equal to = 0.998. Therefore, the oxidation of the MP molecules at the surface of modified electrode, is an adsorption- controlled process.

3.5. The calibration plot and limit of detection

Differential pulse voltammetry was used for quantitative determination of morphine using the HCPE under the optimized experimental conditions. Fig. 4, displays the calibration curve and the inset of this Figure, shows the differential pulse voltammograms of morphine at HCPE. As is shown in this Figure, the peak current increases with increasing the concentration of morphine up to 200 μ M. The calibration curve is linear in the dynamic range of 5 to 200 μ M with a detection limit of 1 μ M. The detection limit was obtained based on the equation of 3 Sb/m, where Sb is the standard deviation of the blank and m is the slope of the calibration curve.

The relative standard deviation (RSD) for 150 μ M of MP solution was found to be: 3.7% for five replicate measurements. The concentration of 150 μ M morphine was also determined at the surface of five fabricated electrode and the RSD was found to be 3.9%. When the electrode was not in use, it was stored at 4 °C in refrigerator. No considerable change was observed in the oxidation peak current of 150 μ M of morphine after storage for 35 days. These results demonstrated the excellent repeatability, good reproducibility and high stability of the fabricated electrochemical sensor.

The analytical performance of HCPE for determination of morphine, with the other reported electrochemical sensors are compared in Table 1. As is evident in this Table, the capability of the proposed electrode is good and comparable with the other sensors. Moreover, an important advantage of the new constructed sensor is simplicity and its ability to measure the concentration of morphine in sub microliter of solution.

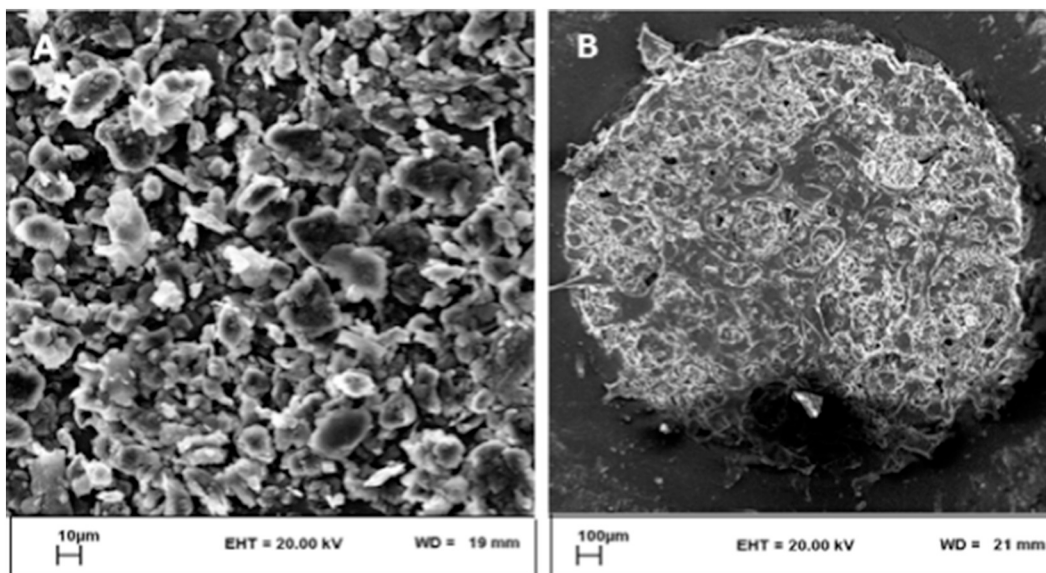


Fig. 1. Scanning electron microscopy images of (A) Unmodified hydrogel carbon paste electrode, (B) Surface modified hydrogel carbon paste electrode with chitosan as a covering film.

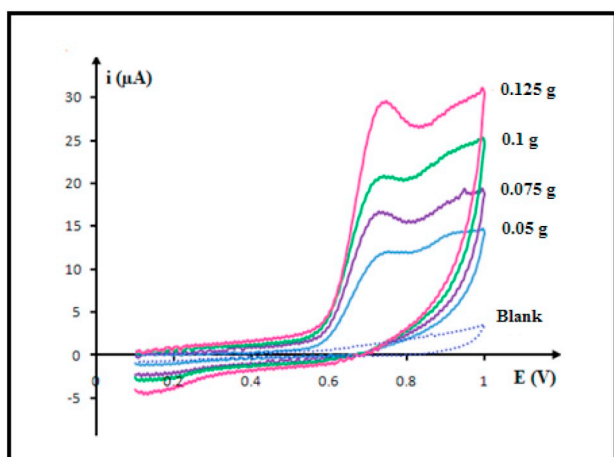


Fig. 2. Cyclic voltammograms of morphine (150 μM , PBS pH 7, Scan rate: 100 mV/s) at hydrogel carbon paste electrodes with different amount of the hydrogel.

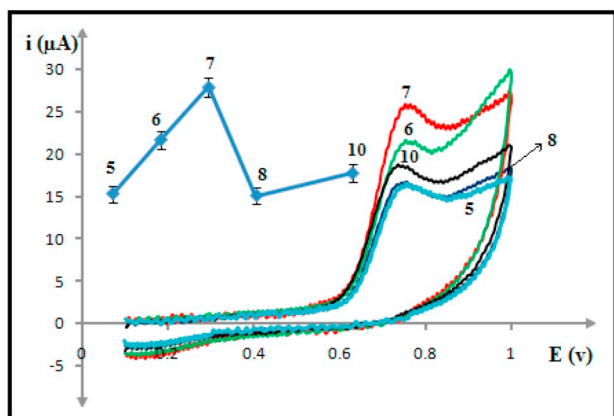


Fig. 3. Cyclic voltammograms of 150 μM morphine solution on the HCPE in various pH values (5.0, 6.0, 7.0, 8.0, and 10.0) at a scan rate of 100 mV/s.

3.6. Interference study

The selectivity of the electrode was investigated by determination of morphine in the presence of some interference compounds such as catechol, ascorbic acid, salicylic acid, glucose, fructose and uric acid. The current response of morphine in the presence of interference (I) was compared with the current in the absence of interference (I^0). The cyclic voltammograms of morphine in the presence of phenolic interferences are shown in Fig. 5. As can be seen from this Figure, catechol and ascorbic acid was also oxidized at the potential of 0.50 and 0.46 V respectively, but the salicylic acid shows no oxidation peak at the electrode surface. The results confirmed that the mentioned phenolic interferences do not alert the current response of morphine. According to the DPV data which are summarized in Table 2, the biological compounds also do not change the current response of morphine. Therefore, the proposed electrode has a high selectivity towards the morphine in solutions.

3.7. Analytical application of the sensor

To assess the practical performance of the electrode, spiked healthy and patient human urine samples were analyzed by standard addition method. The patient human was used cefixime (from FARABI pharmaceutical Co), biocold fort (from BAKHTAR Biosoimi Co) and vitamin C (from MODAVA pharmaceutical Co) 4 h before the urine sampling. Each urine sample was centrifuged and diluted 50 times with PBS (pH 7.0) before standard addition. The obtained results in Table 3 are in good agreement with those which are assigned by the known morphine spiked in the urine samples that demonstrate the utility of the sensor for determination of morphine content in urine sample. In the patient urine sample there was also a distinct oxidation peak before the oxidation peak of morphine which had not any interference with determination of morphine.

4. Conclusion

In this research work, we used the hydrogel as an absorbent modifier to develop a new class of electrochemical sensor with capability of analyte measurement in 0.5 μL of the sample solutions. Differential pulse voltammetric measurements exhibit a linear dynamic ranges of 5–200 μM with a detection limit of 1 μM with respect to the

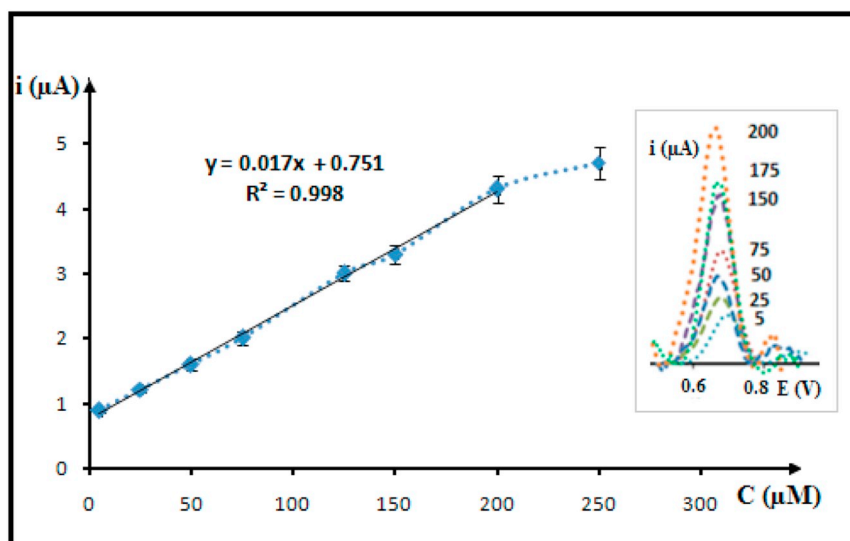


Fig. 4. The plot of current response vs. morphine concentration. Inset shows the differential pulse voltammograms of MP in various concentrations ranging from 5 to 200 μM , with a scan rate of 100 mV/s, electrolyte; 0.1 M phosphate buffer; pH 7.0.

Table 1

Comparison of the electroanalytical data for morphine determination.

Electrodes	Electrochemical method	Linear range (μM)	Detection limit (μM)	Reference
CoHCFe/GCE ^a	DPV	1–50	0.5	[12]
OMC/GCE ^b	CV	0.1–20	0.01	[14]
MWCNTs–Nafion/ GCE ^c	ASV	0.1–4	0.03	[15]
GCE	CV	4–100	0.2	[17]
CdO/NPs/ILs/CPE ^d	SWV	0.5–800	0.1	[20]
HCPE	DPV	5–200	1	Present work

^a Cobalt hexacyanoferrate/glassy carbon electrode.

^b Ordered mesoporous carbon modified glassy carbon electrode.

^c Multi-walled carbon nanotubes/Nafion/glassy carbon electrode.

^d CdO nanoparticles, ionic liquid, carbon paste electrode.

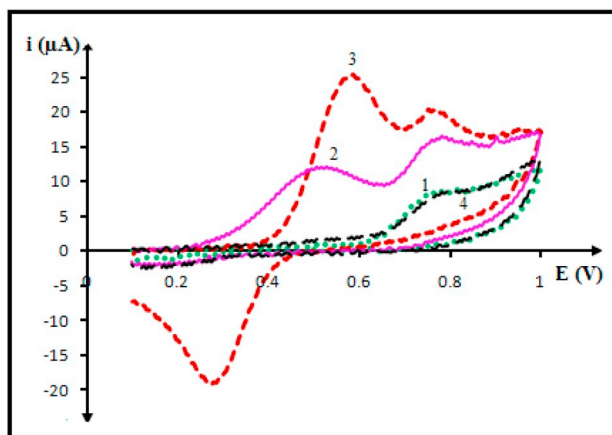


Fig. 5. Cyclic voltammograms of 50 μM of morphine (curve 1) and in the presence of 50 μM Ascorbic acid (curve 2), Catechol (curve 3), Salicylic acid (curve 4) at HCPE in 0.1 M PBS (pH 7). Scan rate: 100 mV/s.

concentration of morphine. Generally, the fabricated electrochemical sensor, possess desirable characteristics such as: simplicity of preparation, easy surface renewal of the electrode, good reproducibility and repeatability, wide linear concentration range, low detection limit, low cost and more importantly the ability of analyte measurement in a small

Table 2

The effect of interferences in determination of morphine, using the hydrogel carbon paste electrode.

Substance (100 μM)	Current ratios (I/I^*) ^a
Catechol	0.95
Salicylic acid	0.99
Ascorbic acid	1.01
Urea	0.95
Fructose	1.05
Glucose	0.97

^a Current ratios for mixtures of 1:1 of interfering substances and morphine with the same concentration (I) compared to that of morphine (I^*).

Table 3

Differential pulse voltammetric determination of morphine in urine samples ($n = 3$). The patient human was used some drug before urine sampling.

Sample	Content (μM)	Added (μM)	Found (μM)	Recovery%	RSD%
Healthy Urine	–	150	152.4	101.6	3.9
Patient Urine	–	150	146.7	97.8	4.1

drop. The modified electrode also successfully applied for the selective determination of morphine in urine matrix.

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