Research Article

Voltammetric Determination of Codeine on Glassy Carbon Electrode Modified with Nafion/MWCNTs

Robert Piech, Martyna Rumin, and Beata Paczosa-Bator

Faculty of Material Science and Ceramics, AGH University of Science and Technology, Mickiewicza 30, 30059 Cracow, Poland

Correspondence should be addressed to Beata Paczosa-Bator; paczosa@agh.edu.pl

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A glassy carbon electrode modified with a Nafion/MWCNTs composite is shown to enable the determination of codeine using differential pulse voltammetry in phosphate buffer of pH 3.0. At a preconcentration time of 15 s, the calibration graph is linear in the 0.5 \( \mu \text{M} \) (0.15 mg L\(^{-1}\)) to 15 \( \mu \text{M} \) (4.5 mg L\(^{-1}\)) concentration range with a correlation coefficient of 0.998. The detection limit at a preconcentration time of 120 s is as low as 4.5 \( \mu \text{g} \cdot \text{L}^{-1} \). The repeatability of the method at a 0.6 \( \mu \text{g} \cdot \text{L}^{-1} \) concentration level, expressed as the RSD, is 3.7% (for \( n = 5 \)). The method was successfully applied and validated by analyzing codeine in drug, human plasma, and urine samples.

1. Introduction

Codeine is a naturally occurring opium alkaloid. It is chemically known as 7,8-didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol monohydrate [1] but is less potent than morphine, with a potency ratio of 1:10 [2]. Codeine is considered as a prodrug, metabolized to active compounds of morphine and codeine-6-glucuronide [3]. It is traditional choice for the treatment of mild and moderate pain [4] and is frequently recommended for pediatric use [5]. Moreover, codeine is widely used in cough cold syrup, but it would cause drug addiction and make mental damage to patient if abused [6]. Thus a sensitive, specific, fast, and cheap method of determining codeine is necessary for studying the presence of codeine in drugs and human body fluids.

The analytical methods most often used to determine codeine in various samples are high performance liquid chromatography [7–9], spectrophotometry [10, 11], gas chromatography [12], and capillary electrophoresis [13]. However, these methods are usually time consuming. Electrochemical methods for the determination of codeine as potentiometry and polarography are also reported [14, 15] but these methods are of rather low sensitivity. In the group of more sensitive methods as voltammetry various working electrodes such as glassy carbon electrode (the detection limit not shown) [16], Nafion/ruthenium oxide pyrochlore CME chemically modified electrode with the detection limit of 10 nM [17], clay-modified screen-printed carbon electrode (CMSPE) with the detection limit of 20 nM [18], Prussian blue film modified-palladized aluminum electrode (PB/Pd/Al) with the detection limit of 0.8 \( \mu \text{M} \) [19], aluminum electrode modified with a thin layer of metallic palladium (Pd/Al) with the detection limit of 5 \( \mu \text{M} \) [20, 21], boron-doped diamond film electrode with the detection limit of 80 nM [22], and single-walled carbon nanotubes modified carbon ceramic electrode (SWCNTs/CCE) with the detection limit of 110 nM [23] are used.

The aim of this work was to study the high sensitive determination of codeine by means of linear sweep voltammetry (LSV) and differential pulse voltammetry (DPV) with the use of glassy carbon (GC) electrode modified with Nafion/multiwalled carbon nanotubes (MWCNTs). The new procedure was examined and successfully used for the determination of a low codeine concentration in urine, human blood plasma, and medical products. Potential interference from selected metal ions, ascorbic acid, citric acid, and surface-active substances was checked.
2. Material and Methods

2.1. Measuring Apparatus and Software. A multipurpose Electrochemical Analyzer M161 with the electrode stand M164 (MTM-ANKO, Poland) was used for all voltammetric measurements. The classical three-electrode quartz cell, volume 20 mL, consists of a GC electrode (diameter 3 mm, Mineral, Poland) modified with Nafton/MWCNTs as the working electrode, a double junction reference electrode Ag/AgCl/KCl (3 M) with replaceable outer junction (3 M KCl), and a platinum wire as an auxiliary electrode. pH measurements were performed with a laboratory pH-meter (N-512 elpo, Polymetron, Poland). Stirring was performed using a magnetic bar rotating at approximately 500 rpm.

All experiments were carried out at room temperature. The stirring was performed (ca. 500 rpm) using a magnetic stirring bar. Then, after a rest period of 5 s a differential pulse voltammogram was recorded in the anodic direction from 300 to 1350 mV. The other experimental parameters were as follows: step potential, 5 mV; pulse potential, 50 mV; time step potential, 40 ms (20 ms waiting + 20 ms sampling time). Measurements were performed in solutions not previously treated with deoxygenation. Quantitative measurements were performed using the standard addition procedure.

2.2. Chemicals and Glassware. All reagents used were of analytical grade. KH$_2$PO$_4$ and K$_2$HPO$_4$ were obtained from Merck and H$_2$PO$_4$ was obtained from CHEMAN (Poland). In measurements a 0.1 M phosphate buffer solution (pH 3.0) was used (prepared using quadruply distilled water).

Standard stock solutions of codeine (0.01 M) were prepared for several measurements. The classical three-electrode quartz cell, volume 20 mL, consists of a GC electrode (diameter 3 mm, Mineral, Poland) modified with Nafton/MWCNTs as the working electrode, a double junction reference electrode Ag/AgCl/KCl (3 M) with replaceable outer junction (3 M KCl), and a platinum wire as an auxiliary electrode. pH measurements were performed with a laboratory pH-meter (N-512 elpo, Polymetron, Poland). Stirring was performed using a magnetic bar rotating at approximately 500 rpm. All experiments were carried out at room temperature. The MTM-ANKO EAGRAPHER software enabled electrochemical measurements, data acquisition, and advanced processing of the results.

2.3. Preparation of the Electrode. Prior to modifying the GC electrode was mechanically polished with Al$_2$O$_3$ (0.05 μm) and then rinsed and sonicated 5 min in distilled water. Next 10 mg of MWCNTs was added to 10 mL ethanol and Nafton (final Nafton concentration 0.1%) and then sonicated for 2 hours to obtain a homogenous suspension. The prepared GC electrode was coated with 10 μL homogenous Nafton/MWCNTs and allowed evaporating the solvent at room temperature in the air. Nafton/MWCNTs GC electrode was conditioned in phosphate buffer (pH 3.0) and can be used for several measurements.

2.4. Standard Procedure of Measurements. The electrochemical behavior of the Nafton/MWCNTs glassy carbon modified electrode was investigated using cyclic voltammetry. The voltammograms were recorded in the potential range from 875 to 1425 mV. Before each registration scan the potential of 1450 mV (2 s) was applied to clean the surface of the electrode. The electrode conditioned in this way was used to determine codeine in the supporting electrolyte: 0.1 M phosphate buffer (pH 3.0) (total volume 10 mL) contained in a quartz voltammetric cell. The anodic peak potential was shifted in the positive direction with the increasing scan rate. The peak potential versus ln scan rate gave a straight line (Figure 2).

The obtained linear regression equation is

$$E_p = 28.4 \ln (v) + 1060 \text{[mV]}, \quad r = 0.997. \quad (2)$$

Based on the theory for an irreversible electrode reaction [24] from the slope of $E_p$ versus ln$v$, $\alpha n = 0.45$ could be obtained.
3.2. Influence of DPV Parameters Technique on Codeine Peak.

The important parameters of the DPV technique are pulse amplitude (|Δ𝐸|), potential step amplitude (|𝐸_s|), waiting time (𝑡_w), and sampling time (𝑡_s). Consequently, these parameters were investigated. To optimize the conditions for codeine measurements, the following instrumental parameters were systematically varied: Δ𝐸 in the range 5−100 mV (both positive and negative modes), 𝐸_s in the range 1−7 mV, and 𝑡_w and 𝑡_p from 10 to 60 ms.

The best results were obtained for the amplitude of 50 mV (the peak current was ∼12 μA for 10 μM codeine, Figure 3). Higher pulse amplitude (>50 mV) caused the growth of the background current practically without increase of the peak current. For further work, the pulse amplitude of 50 mV was applied.

Changes of the step potential cause influence on peak current. For a step potential equal to 1 mV the peak current was 3.8 μA, and for a step potential of 7 mV the peak current was 14 μA (Figure 3). The step potential of 5 mV was applied in further work (good relation codeine signal to background current).

The waiting time and sampling time were changed in the range from 10 to 60 ms (Figure 4). The best result was
3.3. Influence of the Volume of Nafion/MWCNTs. The mixture of Nafion/MWCNTs coating the GC electrode is necessary to obtain a high sensitive determination of codeine. The codeine peak current depends on the volume of Nafion/MWCNTs (Figure 5).

For bare GC electrode the codeine peak current was 0.3 µA. Presence and increase of the amount of Nafion/MWCNTs on the GC electrode are accompanied by an increase of the codeine peak. The optimal volume of Nafion/MWCNTs was 10 µL (with the peak current reaching values about 12 µA). Higher volumes of Nafion/MWCNTs cause increase in a background current and worse repeatability of the codeine signal. The presence of Nafion/MWCNTs also had an influence on the peak potential. For bare GC electrode the DPV codeine peak potential was 1180 mV and for modified electrode with Nafion/MWCNTs the codeine peak potential was 1125 mV. The negative shift of the peak potential suggests catalytic effect caused by Nafion/MWCNTs. For further work, the volume of 10 µL was used.

3.4. Influence of Preconcentration Potential and Time on Codeine Peak. The influences of preconcentration potential and time are usually important factors on the sensitivity and detection limit of the stripping methods. Preconcentration potential for codeine determination in 0.1 M phosphate buffer (pH 3.0) was investigated in the range from –200 to 900 mV. The experiment showed that the preconcentration potential has no influence on the codeine peak current. In the whole work arbitrary the 300 mV preconcentration potential was used.

The changes in magnitude of the codeine current versus preconcentration time are presented in Figure 6.

The peak current increased with the increase of the preconcentration time from 1.9 µA ($t_{acc} = 0$ s) to 24.3 µA ($t_{acc} = 480$ s). For a preconcentration time higher than 120 s, practically no increase of the codeine peak current was observed. The codeine peak potential is independent of the preconcentration potential.

3.5. Influence of Electrolyte Composition and pH on Codeine Peak. The electrochemical oxidation of codeine has been studied in 0.1 M KCl (pH 6.8), KNO$_3$ (pH 6.9), acetate buffer...
The DPV voltammograms of codeine for the 0.05–0.35 μM concentration range and preconcentration time of 120 s are presented in Figure 8. The detection limit obtained for short preconcentration time (15 s) was 130 nM with the linearity up to 15 μM (slope of the regression line was 1.02 ± 0.03 (μA·μM⁻¹), intercept 0.21 ± 0.23 μA, and correlation coefficient 0.998). A longer preconcentration time results in a lower detection limit (e.g., when the preconcentration time of 60 s was used during the measurement the detection limit was 22 nM, and for the preconcentration time of 120 s the detection limit was 14 nM). The slopes for regression lines were (μA·μM⁻¹) 2.27 ± 0.02 and 3.67 ± 0.05, intercepts (μA) 0.21 ± 0.22 and 0.04 ± 0.03, and the correlation coefficients 0.996 and 0.997 for preconcentration times of 60 and 120 s, respectively. The linearity was up to 1.75 μM (tacc = 60 s) and 0.5 μM (tacc = 120 s).

To validate the method the urine, human plasma, and drugs were investigated. The samples, spiked with codeine, were analyzed according to the described procedure using the GC electrode modified with Nafion/MWCNTs. Determinations of codeine were performed using the standard addition method (three additions of the standard solution). Results from codeine determination are presented in Table 1. The recovery of codeine ranged from 92 to 105%. The analytical usefulness of the presented method for the determination of codeine in the samples was confirmed.

4. Conclusions
The presented DPV method for the electrochemical determination of codeine using a GC electrode modified with Nafion/MWCNTs allows determining codeine at trace level,
Table 1: Results of codeine determination in various samples.

| Codeine added | Urine (mg/L) | Human plasma (mg/L) | Syrup¹ (mg/g) | Tablet² (mg/tablet) |
|---------------|-------------|---------------------|-------------|-------------------|
| 0             | 0           | 0                   | 15.5 ± 0.7  | 15.3 ± 0.4        |
| 0.15 mg/L     | 0.144 ± 0.009 (96) | 0.138 ± 0.012 (92) | —           | —                |
| 0.6 mg/L      | 0.632 ± 0.027 (105) | 0.57 ± 0.03 (95) | —           | —                |
| 15 mg         | —           | —                   | 31.4 ± 1.1 (103) | 30.9 ± 0.9 (102) |

¹Product declared 15 mg/10 mL.
²Product declared 15 mg/tablet.

Table 2: Voltammetric detection of codeine reported at various electrodes.

| Electrode | Linear range (µM) | Detection limit (nM) | Reference |
|-----------|-------------------|----------------------|-----------|
| CME       | 0–32              | 10                   | [17]      |
| CMSPE     | 2.5–45            | 20                   | [18]      |
| PB/Pd/Al  | 2–30              | 10–30                | [19]      |
| Pd/Al     | 0.1–3             | 5                    | [20]      |
| Boron-doped diamond | 0.1–60 | 80                  | [22]      |
| SWCNTs/CCE | 0.2–230         | 0.11                 | [23]      |
| Nafion/MWCNTs \(t_{acc} = 120 \text{s}\) | 0–0.5 | 14                 | This work |

in concentrations as low as 14 nM \((4.5 \mu g \cdot L^{-1})\), calculated according to [25] for a preconcentration time of 120 s. The reproducibility of the method is very good; that is, when measured as RSD it is 3.7%. Acceptable recovery (92–105%) shows that the method can be used for the determination of codeine in drugs and human body fluids.

The preparation of GC electrode modified with Nafion/MWCNTs is very simple, short, and economically acceptable. The obtained results confirm that method may be used in out-of-laboratory systems.

Voltammetric responses of Nafion/MWCNTs in terms of linear range and detection limits were compared to the other electrodes reported in the literature (Table 2).

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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