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Voltammetric Oxidation of Drugs of Abuse III. Heroin and Metabolites

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Abstract

The oxidative behavior of heroin in aqueous solution is reported. In order to identify its oxidation peaks, several metabolites, 6-monoacetylmorphine, 3-monoacetylmorphine and norheroin, were synthesized and their electrochemical behavior studied using differential pulse voltammetry. The anodic waves observed for heroin correspond to the oxidation of the tertiary amine group and its follow-up product (secondary amine), and to the oxidation of the phenolic group obtained from hydrolysis, at alkaline pHs, of the 3-acetyl group. The results enabled a new oxidative mechanism for heroin to be proposed in which a secondary amine, norheroin, and an aldehyde are obtained. The voltammetric behavior of 6-monoacetylmorphine and morphine was found to be similar demonstrating that the presence of an acetyl substituent on the 6-hydroxy group does not have a relevant influence on the peak potential of the wave resulting from oxidation of the 3-phenolic group.

Keywords: Heroin, 3-Monoacetylmorphine, 6-Monoacetylmorphine, Norheroin, Oxidation, Voltammetry, Drugs of abuse

1. Introduction

Heroin (3,6-diacetylmorphine, diamorphine) is a potent synthetic opiate analgesic processed from morphine, a naturally occurring substance extracted from the seed pod of certain varieties of poppy plants. Although heroin was first introduced into medicine in 1898 as a cough suppressant, it was soon banned because of its high addictive character [1].

Today, heroin is still the prime indicator for problem drug abuse. It is both the most abused and the most rapidly acting of the opiates. The use of heroin continues to increase and is estimated that eight million people in the world abuse opiates [2].

The increase of chemical studies on heroin has been motivated by its pharmacological significance, in the hope that a better understanding of its biological behavior at the molecular level could result in the development of compounds with lesser abuse capacity and the same therapeutic activity.

The widespread use of heroin as an illicit drug has led to an increased effort towards developing and improving methods for its determination in seizure and in biological samples. Aqueous solutions of heroin are much less stable than solutions of other opiates such as morphine or codeine. In fact, this drug is rapidly deacetylated in aqueous solution at alkaline pH to form 6-monoacetylmorphine (6-MAM) and is further slowly hydrolyzed to form morphine [3, 4].

Moreover, in man heroin is also rapidly metabolized to 6-monoacetylmorphine and further to morphine [5]. Hence, due to its instability, the methods developed should also be appropriate for the determination of its breakdown products or metabolites.
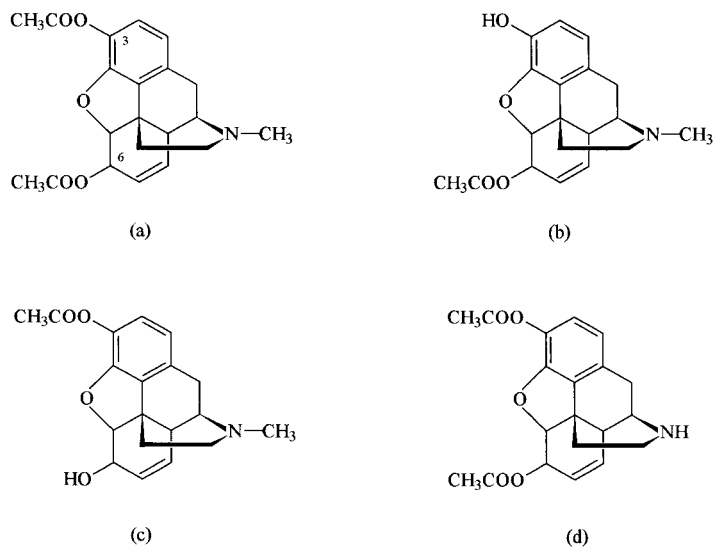

Fig. 1. Structural formulae of a) heroin, b) 6-monoacetylmorphine, c) 3-monoacetylmorphine and d) norheroin.

Several chromatographic procedures are currently available for determination of heroin and metabolites resorting to the suitability and usefulness of electrochemical detection for the analysis of trace levels of drugs, due to its high sensitivity [6–8]. A voltammetric assay, using a carbon paste electrode, was also proposed for determination of heroin in illicit dosage forms [9]. From this study, a mechanism for the electrochemical oxidation of the drug was also postulated.

In order to clarify some inconsistent results presented in the literature a thorough, detailed study of the electrochemical behavior of heroin has been performed. Moreover, to support and clarify all the oxidation processes occurring and to identify the products formed, the synthesis of several heroin metabolites, 6-monoacetylmorphine, 3-monoacetylmorphine and norheroin, (Fig. 1) as well as the study of their electrochemical behavior was carried out.

2. Experimental

2.1. Apparatus

Voltammetric measurements were performed using an Autolab PGSTAT 10 potentiostat/galvanostat (EcoChimie, Netherlands) and a one-compartment glass 663 VA Metrohm cell with a three-electrode configuration (Metrohm). The electrodes used were a glassy carbon working electrode with a diameter of 2 mm (Metrohm), a glassy carbon rod counter electrode (Metrohm) and an Ag/AgCl (3 M KCl) reference electrode (Metrohm). The working electrode was polished with alumina (BDH) on a microcloth pad and rinsed with water before use.

A Metrohm E-520 pH-meter and a Metrohm glass electrode were used for pH measurements.

Melting points were measured using a Köfler microscope (Reichert Thermovar). Infrared spectra were recorded on an ATI Mattson Genesis Series FTIR spectrophotometer using potassium bromide disks (Uvasol, Merck). ^{1}H and ^{13}C NMR (^{1}H decoupled) data were acquired, at room temperature, on a Brüker AMX 300 spectrometer operating at 300.13 and 75.47 MHz, respectively. Electron impact mass spectra (EI-MS) were performed on a VG AutoSpec instrument.

2.2. Other Conditions

Thin-layer chromatography (TLC) was carried out on pre-coated silica gel 60 F254 (E. Merck). The layer thickness was 0.2 mm. The following chromatographic systems were used: chloroform/methanol/formic acid (9:1:0.1), chloroform/acetic acid/diethylamine (8:1.5:0.5), chloroform/methanol (9:1), chloroform/methanol/diethylamine (8:1.5:0.5), chloroform/methanol/diethylamine (9:1:0.5), carbon tetrachloride/butanol/methanol/ammonia (4:3:3:0.3), methanol/ethyl acetate (7.5:2.5). The spots were visualized under UV detection (254 and 366 nm) and iodine vapor. Solvents were evaporated in a Büchi Rotavapor.

2.3. Reagents and Solutions

Morphine hydrochloride was obtained from Uquipa (Lisbon, Portugal) and was used without further purification. Normorphine was prepared as described elsewhere [10]. Reagents used in the synthetic procedures were obtained from Sigma-Aldrich Quimica (Sintra, Portugal). All other reagents and solvents were *pro analysis* grade and purchased from Merck (Lisbon, Portugal). Deionized water with conductivity less than 0.1 µS cm^{-1} was used throughout. Buffer solutions employed were 0.2 M in the pH range 1.2–12.2 [11].

2.3.1. Synthesis of Heroin (Diacetylmorphine, Diamorphine, Acetomorphine, 3,6-O-Diacetylmorphine)

The synthetic procedure used to prepare heroin was adapted from the literature [12–14].

Morphine hydrochloride (0.5 g) was dissolved in a mixture of 15 mL of pyridine and 20 mL of acetic anhydride. The solution was stirred, at room temperature, during 24 hours and was then poured in ice, washed with 10% sodium bicarbonate solution and extracted with chloroform (3 × 50 mL). The organic layers were combined, washed with water and dried over anhydrous magnesium sulfate. The solvent was partially evaporated and diethyl ether was added. After cooling, the white solid was filtered, washed and dried in an oven.

The analytical data obtained for heroin were similar to those found in literature [15, 16].

2.3.2. Synthesis of 3-Monoacetylmorphine (3-MAM; 3-O-Acetylmorphine)

The synthesis of 3-monoacetylmorphine was adapted from procedures described in the literature [12, 17]. To a stirred suspension of morphine base (0.5 g) in 50 mL of a 10% aqueous sodium bicarbonate solution, 2.5 mL of acetic anhydride was added dropwise. The mixture was extracted with chloroform (2 × 50 mL). The organic phases were combined, washed with water and dried over anhydrous magnesium sulfate. The solvent was then removed under reduced pressure to obtain a viscous oil.

The analytical data obtained for 3-monoacetylmorphine were similar to those found in the literature [16, 17].

2.3.3. Synthesis of 6-Monoacetylmorphine (6-MAM; 6-O-acetylmorphine)

The synthetic procedure was adapted from the literature with slight modifications [13].

Heroin (0.15 g) was dissolved, using an ultrasonic bath, in 20 mL of ethanol and 0.3 g of hydroxylammonium chloride was added. The reaction was stirred, at room temperature, during 20 hours. After complete evaporation of the solvent the residue was dissolved in 40 mL of saturated sodium bicarbonate solution. After extraction with chloroform (3 × 50 mL) the organic phases were combined, washed with water and dried over anhydrous sodium sulfate. After

solvent evaporation, an oily product was obtained which was crystallized from diethyl ether. The hydrochloride salt was obtained by dissolving the base in ethanol and adding concentrated hydrochloric acid. A white solid was obtained, filtered, washed with ethyl ether and dried in an oven.

The analytical data obtained were similar to those found in the literature [12, 15, 16, 18].

2.3.4. Synthesis of Norheroin (Desmethylheroin)

Normorphine was synthesized following a previously described procedure [10]. Normorphine was acetylated using acetic anhydride and pyridine. After the usual workup, purification was done by preparative TLC (silica gel; chloroform/methanol (9:1)). After extraction with chloroform the residue was crystallized from petroleum benzine/diethyl ether.

The structural data of norheroin were in accordance with those reported in the literature [19].

3. Results and Discussion

The chemical breakdown of heroin, mostly due to hydrolysis, produces primarily 6-monoacetylmorphine (6-MAM) along with small amounts of 3-monoacetylmorphine (3-MAM) and morphine. The rate of hydrolysis is strongly influenced by parameters such as temperature and pH [4].

In order to establish the oxidative mechanism of heroin several metabolites were synthesized: 6-monoacetylmorphine, 3-monoacetylmorphine and norheroin, as described in Section 2.

3.1. Electrochemical Oxidation

The electrochemical behavior of heroin was studied over the pH interval 1.2 to 12.2, at a glassy carbon working electrode using differential pulse voltammetry (Figs. 2a and 2b). The results obtained led to the conclusion that the anodic oxidation of heroin is a simple process compared to that of codeine and morphine and, as expected, that it is highly influenced by pH. A single anodic wave is seen above pH 3, $E_p = +1.20$ V, corresponding to the oxidation of the tertiary amine group. A second peak is seen at pH 8 and above, $E_p = +1.08$ V, and occurs as a result of the oxidation of the secondary amine formed by oxidation of the tertiary amine group present in the heroin molecule (Figure 1). The third peak, starting at pH 10, $E_p = +0.27$ V, corresponds to the oxidation of the phenolic group resulting from the hydrolysis of heroin to 6-MAM (Scheme 1).

The anodic wave observed at $pH \geq 3$, $E_p = +1.20$ V, where the peak potential decreases as the pH increases (Fig. 2b), can easily be attributed to the oxidation of the tertiary amine group, by comparison with the voltammetric behavior of the heroin metabolites studied, in particular norheroin, as well as the behavior of other opiate analogues morphine and codeine (data not presented) [10]. The results
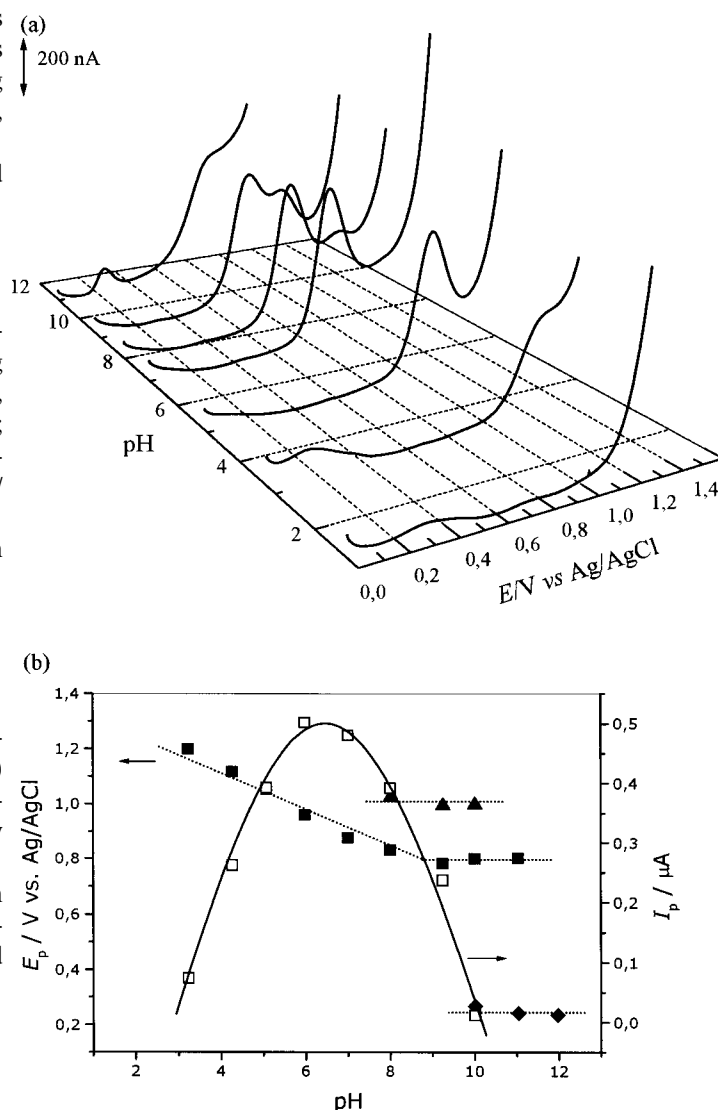

Fig. 2. a) 3D plot and b) plots of E_p (filled symbols) and I_p (open symbols) vs. pH from differential pulse voltammograms of 100 μM heroin in different buffer electrolytes as a function of pH. Scan rate 5 mV s^{-1}.

obtained using a glassy carbon electrode are partially in agreement with those reported in the literature [9]. In fact, using a carbon paste electrode, a single wave was observed for all pHs and the peak was also related to the oxidation of the aliphatic tertiary amine. Nevertheless, the mechanism proposed therein involved the formation of a secondary amine, other than norheroin, and a ketone.

The new results presented in this paper, using a glassy carbon electrode, clearly indicate a different mechanism for the oxidation of the tertiary amine group. They show the formation of a secondary amine (norheroin) and an aldehyde (Scheme 1) which is in accordance with the data described in the literature for the oxidation of aliphatic tertiary amines [20, 21].

The second anodic wave which begins to appear at pH 8, $E_p = +1.08$ V, is due to further oxidation of the product
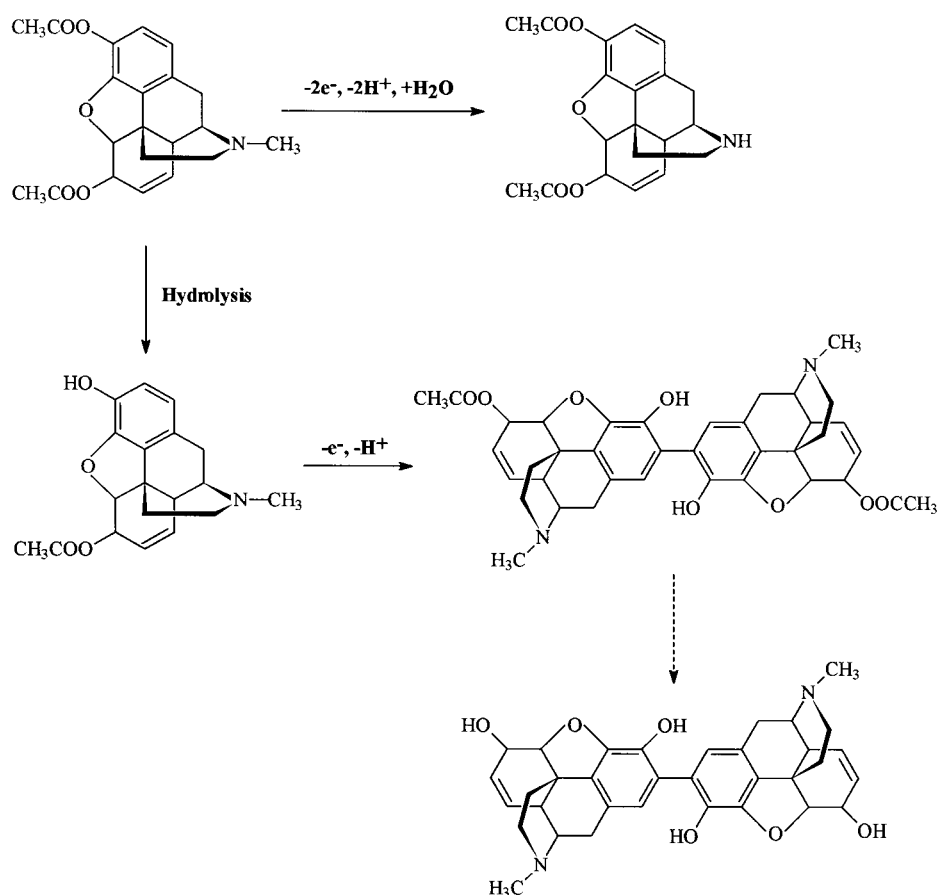
Scheme 1.

resulting from the aliphatic tertiary amine oxidative process. Evidence for this comes from the voltammetric study of norheroin (Fig. 3d). As seen, the peak observed for heroin at pH 8 is coincident with that observed for norheroin at the same pH (Fig. 4). The appearance of a second peak being attributed to further oxidation of the secondary amine formed by oxidation of the aliphatic tertiary amine group has been described in the literature for other aliphatic amines [20].

The third peak appearing as a shoulder at pH $\geq$ 10, E_p = + 0.27 V, but only being well-defined at pH 11, can be assigned to the oxidation of the phenolic group resulting from the hydrolysis of heroin (Scheme 1). This conclusion seems obvious keeping in mind that in alkaline solution this drug is, rapidly deacetylated to form 6-monoacetylmorphine (6-MAM). The voltammetric study of 6-MAM and 3-MAM provides evidence to confirm this hypothesis (Fig. 3b and 3c). 6-MAM presents two anodic waves at pH 11, one resulting from the oxidation of the tertiary amine group, and the other from an oxidation process involving the phenolic group present in the molecule. Comparing the results obtained for heroin, 3-MAM and 6-MAM in alkaline solution it is easy to attribute the third wave to oxidation of the phenolic group resulting from hydrolysis of the 3-acetyl group (Fig. 5).

The existence of some discrepancy in the literature between the potentials at which the phenolic group is oxidizable in 6-MAM and in morphine led us to study and compare their voltammetric behavior (Fig. 6). It is mentioned that the optimal detection potential necessary to determine 6-MAM at pH 6.5 is ca. 150 mV higher than that of morphine this difference being attributed to the presence in the first of an acetyl group instead of the 6-hydroxy group [7]. Nevertheless, it seems that this difference, or at least of this magnitude, could not be attributed to the presence of a 6-acetyl group in 6-MAM. In fact, the voltammetric behavior of 6-MAM at pH 7 closely resembles that observed for morphine.

4. Conclusions

The oxidative behavior of heroin in aqueous solution was studied in detail and was found to be simpler than that of codeine and morphine and dependent on pH. In order to understand and clarify some inconsistant data found in the literature several metabolites, 6-monoacetylmorphine, 3-monoacetylmorphine and norheroin, were synthesized and studied.

It was concluded that the anodic waves observed for heroin are related to the oxidation of the tertiary amine group and its resulting product (secondary amine) and to the oxidation of the phenolic group obtained from hydrolysis, at alkaline pHs, of the 3-acetyl group.
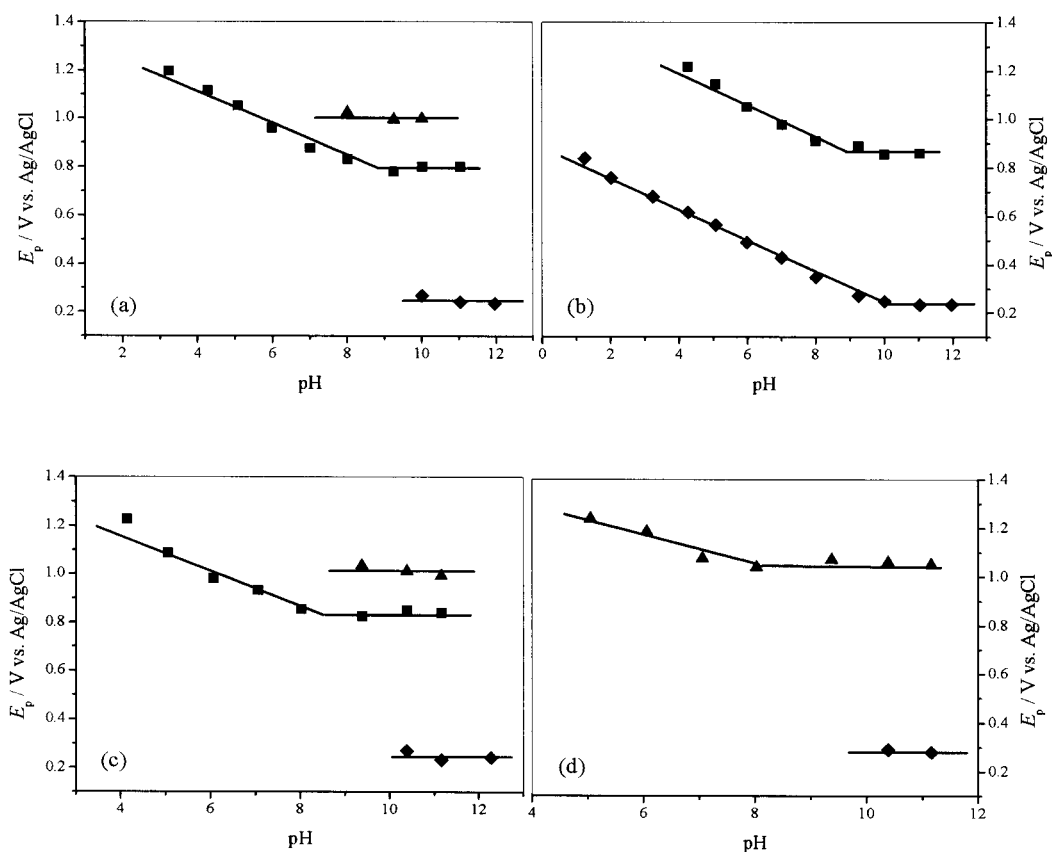
Fig. 3. Plots of E_p vs. pH from differential pulse voltammograms of 100 μM solutions of a) heroin, b) 6-monoacetylmorphine, c) 3-monoacetylmorphine and d) norheroin in different buffer electrolytes. Scan rate 5 mV s^{-1}. (—) Slope 59.2 mV/pH unit.
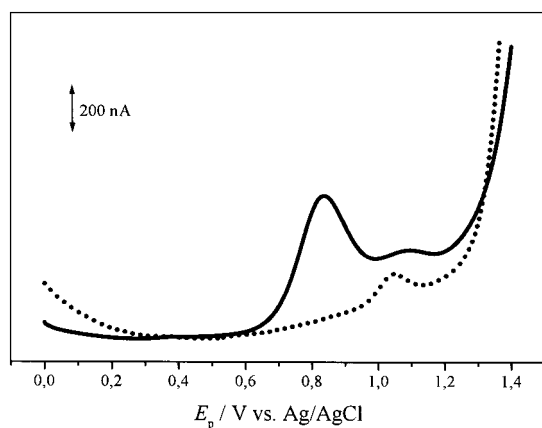

Fig. 4. Differential pulse voltammograms in pH 8 phosphate buffer of 100 μM solutions: (—) heroin and (·······) norheroin. Scan rate 5 mV s^{-1}.
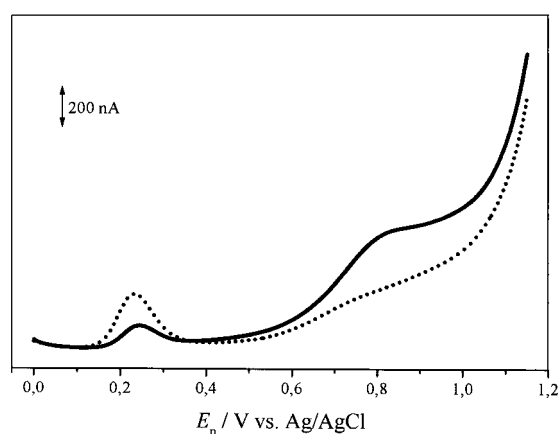

Fig. 5. Differential pulse voltammograms in pH 11 K$_2$HPO$_4$/NaOH buffer of 100 μM solutions: (—) heroin and (·······) 6-monoacetylmorphine. Scan rate 5 mVs^{-1}.

A new oxidative mechanism for heroin was proposed, being more consistent with that described in the literature for the oxidation of aliphatic tertiary amines. The voltammetric behavior of 6-monoacetylmorphine and morphine were compared and verified to be similar, demonstrating that the presence of an acetyl substituent (6-MAM) on the 6-

hydroxy group (morphine) has no visible influence on the peak potential of the wave resulting from oxidation of the 3-phenolic group.
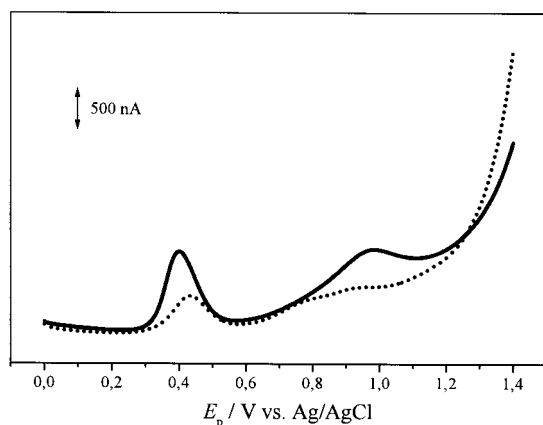
Fig. 6. Differential pulse voltammograms in pH 7 phosphate buffer of 100 μM solutions: (——) morphine and (········) 6-monoacetylmorphine. Scan rate 5 mV s^{-1}.

5. Acknowledgement

6. Supporting Information Available

NMR, IR and EI-MS data of the synthesized compounds is available upon request from the authors.

7. References

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