Full Paper

Voltammetric Oxidation of Drugs of Abuse I. Morphine and Metabolites

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Abstract
A detailed study of the electrochemical oxidative behavior of morphine in aqueous solution is reported. Through the synthesis of several metabolites and derivatives, pseudomorphine, morphine *N*-oxide, normorphine, dihydromorphine and 2-(*N*,*N*-dimethylaminomethyl)morphine, and their voltammetric study it was possible to identify the oxidation peaks for morphine. The anodic waves are related with the oxidation of phenolic and tertiary amine groups. It is also possible to verify that a poorly defined peak observable during morphine oxidation is not a consequence of further oxidation of pseudomorphine but due to formation of a dimer during phenolic group oxidation. The results obtained and especially those regarding the formation of a new polymer based on a C–O coupling could be useful for clarifying the discoloration phenomenon occurring during storage of morphine solutions as well as leading to a better understanding of its oxidative metabolic pathways.

Keywords: Morphine, Pseudomorphine, Morphine *N*-oxide, Normorphine, Dihydromorphine, 2-(*N*,*N*-Dimethylaminomethyl)morphine, Oxidation, Voltammetry, Drugs of abuse

1. Introduction

The use of medicinals by humans is as old as the human race itself, since the need to find measures to combat sickness was as important as the need for food and shelter. However, some of these ancient drugs are also among those that pose serious problems in our contemporary culture through their extensive use for nonmedical purposes. Since these abused substances could turn into a substantial risk to the user and to those with whom he or she may interact, extensive controls have been placed upon the distribution, possession and use of these substances. In addition, detailed chemical and metabolic studies, of a natural or synthetic narcotic, were found to be crucial for both preventive and repressive purposes.

A common aspect of drug metabolism is the existence of one or more oxidative pathways that convert the native molecular form of the compound into reactive species capable of chemically modifying vital cellular constituents [1]. The knowledge of these intermediate metabolites and the investigation of their reactivity is therefore very important.

In this context, electrochemical techniques could play a very important part since besides allowing the detection of low levels of drugs and metabolites in biological fluids such as plasma and urine they could also be used for in vitro studies of the metabolic pathways of these compounds.

Among the commonly encountered types of drugs of abuse, some of the most important are opium alkaloid drugs and their derivatives. Commonly used as therapeutic agents, a number of these compounds are also frequently abused as illicit drugs. The most abundant and economically important opium alkaloid is morphine (Fig. 1). The stability of morphine in aqueous solution has been extensively investigated [2]. It is generally accepted that several factors including oxygen from the air, sunlight and some organic impurities can catalyze the degradation of morphine. It is already known that morphine degrades by oxidation and that the most important products are pseudomorphine and morphine *N*-oxide [2].

The oxidative electroactivity of morphine has been studied by voltammetry using platinum, graphite and glassy carbon electrodes [3–5]. It has been determined that the phenolic group present is responsible for its electroactivity.

The existence of some gaps in knowledge regarding the oxidative behavior of morphine prompted the detailed study described in this paper. In order to identify the oxidation processes and the products formed, several morphine derivatives were synthesized and their electrochemical behavior studied, pseudomorphine, morphine *N*-oxide, normorphine and 2-(*N*,*N*-dimethylaminomethyl)-morphine (Fig. 1).

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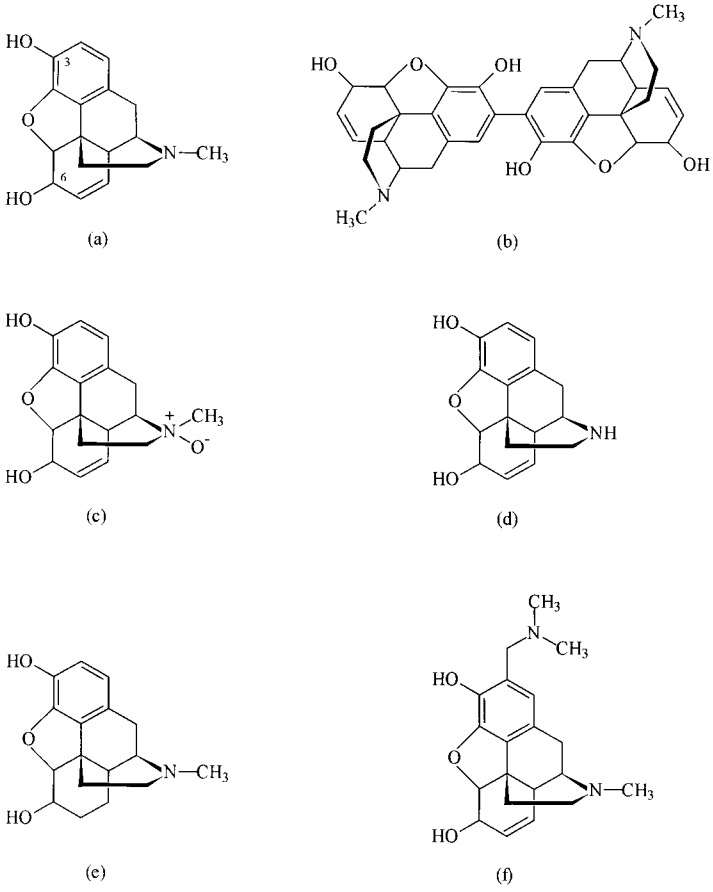


Fig. 1. Structural formulae of a) morphine, b) pseudomorphine, c) morphine *N*-oxide, d) normorphine, e) dihydromorphine and f) 2-(*N*,*N*-dimethylaminomethyl)morphine.

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 obtained on a VG AutoSpec instrument.

2.2. Other Conditions

Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F254 and cellulose plates (E. Merck). The layer thickness was 0.2 and 0.1 mm, respectively. The following chromatographic systems were used: n-butanol/water/acetic acid (4:1:1), water/methanol/acetic acid (8:2:0.1), acetone/water/ammonia (8:1.5:0.5), chloroform/methanol/diethylamine (8:1.5:0.5), carbon tetrachloride/butanol/methanol/ammonia (4:3:3:0.3), chloroform/methanol (9:1), dichloromethane/diethylamine (9:1). The spots were visualized under UV detection (254 and 366 nm) and iodine vapor. Purification of the compounds was performed by column chromatography using Merck silica gel 60 (0.2 – 0.5 and 0.040 – 0.063 mm, E. Merck) or aluminium oxide, activated basic, Brockmann I (ca. 150 mesh, 58 Å, Aldrich). 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. Dihydromorphine hydrochloride was kindly donated by the United Nations Drug Control Program (Vienna, Austria). 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). *m*-Chloroperbenzoic acid (free of *m*-chlorobenzoic acid) was purified according the procedure of Bortolini et al. [6]. 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 [7].

2.3.1. Synthesis of Pseudomorphine (2,2′-Bimorphine; Oxydimorphine)

The synthetic procedure was adapted from the literature [8 – 10].

Morphine hydrochloride (1.0 g) was dissolved, with heating, in 200 mL of 20% potassium hydroxide. After cooling, 40 mL of a 3% potassium ferricyanide solution was slowly added dropwise. The reaction was stirred during 30 min. and refrigerated overnight. The solid obtained was filtered and washed with warm methanol. In TLC analysis pseudomorphine appears as a fluorescent spot [11 – 14].

Due to the low solubility of the compound in water, the sulfate salt was prepared by dissolving the free base in warm glacial acetic acid and adding sulfuric acid until the formation of a precipitate. The product was filtered and recrystallized from water. The compound was filtered, thoroughly washed with water, then with ethyl ether and dried under vacuum.

The analytical data obtained for pseudomorphine was similar to that found in the literature [10, 11, 13, 15].

2.3.2. Synthesis of Morphine N-oxide (Morphine Oxide)

Morphine free base was first prepared in order to be used in the subsequent preparation of morphine *N*-oxide. Morphine hydrochloride (1.0 g) was dissolved in 25 mL of water containing a trace of sodium metabisulfite at 10 °C. One equivalent of concentrated aqueous ammonia was added dropwise, with stirring. The cooling was continued for an additional 15 min. and during this time a white solid appeared in the solution. The crystals were filtered and washed with ice-cold water.

The synthesis of morphine *N*-oxide was then carried out using two different procedures adapted from the literature:

1) Morphine base (0.6 g) was added to 1.5 mL of an ice-cooled 30% hydrogen peroxide solution. The slurry was stirred during 15 min. and then was slowly heated in a water bath till complete dissolution. Acetone was added to cause precipitation. The solid obtained was filtered, washed with acetone and dried in an oven [16, 17].
2) Alternatively morphine *N*-oxide was synthesized using *m*-chloroperbenzoic acid as oxidant as proposed by Craig and Purushothaman [18]. A solution of *m*-chloroperbenzoic acid (0.25 g) in 5 mL of chloroform was gradually added, at 0–5 °C, to an ice-cooled stirred solution of morphine (0.2 g) in 5 mL of chloroform/methanol (1:1). Stirring was continued for 6 hours, during which time the mixture was allowed to warm till room temperature. The solvent was evaporated and the residue obtained was purified by column chromatography (alkaline alumina; chloroform → chloroform/methanol 7:3).

TLC analysis of the product obtained by both methods revealed the presence of two morphine *N*-oxide isomers in different proportions. Before purification, the compounds were converted into the sulfate salt form. After preparative TLC the major isomer was isolated (silica gel; chloroform/methanol/diethylamine (8:1.5:0.5)). The analytical data of the compound were similar to those found in the literature [16, 17, 19, 20].

2.3.3. Synthesis of Normorphine (Desmethylmorphine)

Normorphine was synthesized following a previously described procedure [21]. To a suspension of morphine (0.5 g) and sodium bicarbonate (2.38 g) in 50 mL of chloroform, phenylchloroformate (2.16 g) was added. The mixture was refluxed for 60 hours. The solvent was partially evaporated and 30 mL of water was added. After acidification with concentrated hydrochloric acid, the mixture was extracted with chloroform (3×50 mL). The organic extracts were combined, washed with water, dried over anhydrous sodium sulfate and concentrated. A volume of 20 mL of 95% hydrazine was added to the crude product and the solution was refluxed under nitrogen for 60 hours. After cooling, 20 mL of water was added and washed with ethyl acetate (2×50 mL). The aqueous phase was acidified till pH 2 with concentrated hydrochloric acid. The product was filtered and redissolved in water. After alkalinization with concentrated aqueous ammonia a precipitate was formed, filtered and washed with water. The compound has the same chromatographic behavior as normorphine standard.

2.3.4. Synthesis of 2-(N,N-Dimethylaminomethyl)morphine (2-(N,N-dimethylaminomethyl)-4,5-α-epoxy-17-metyl-morphin-7-en-3,6-α-di ol)

This synthesis was adapted from a reported procedure with slight modifications [22]. Morphine (0.58 g) was dissolved in 30 mL of anhydrous acetonitrile and *N*,*N*-dimethylmethyleneiminium chloride (0.4 g) was added to the solution. The mixture was refluxed for 12 hours. After evaporation of the solvent, the remaining residue was purified by flash chromatography (alkaline alumina; dichloromethane/diethylamine 9:1).

The analytical data were similar to those referred in the literature [22].

3. Results and Discussion

The degradation of morphine in aqueous solution was extensively studied since discoloration occurs during storage of morphine solutions. It is already known that the degradation process is mainly dependent on the pH of the solution and on the presence of atmospheric oxygen [2]. Although some of the morphine degradation products are already known, involving oxidation to pseudomorphine and morphine *N*-oxide, the yellowish to brown discoloration of morphine solutions occurring during storage remains unexplained.

With the aim of clarifying and proving the oxidative mechanism of morphine, several specific congeners were synthesized: pseudomorphine, morphine *N*-oxide, normorphine and 2-(*N*,*N*-dimethylaminomethyl)morphine, as described in the experimental section. The selected compounds possess important functionalities that could help in understanding the oxidative profile of morphine.

3.1. Electrochemical Oxidation

In order to clarify the oxidative pathways of morphine, its electrochemical behavior was studied over a wide pH range (between 1.2 and 12.2) at a glassy carbon working electrode (GCE) using differential pulse voltammetry (Figs. 2a and 2b). Excluding the broad peak observed at +0.3 V from pH 1 to 3, that is related with the GCE background current, two well-defined anodic peaks can be observed. The first peak starting at pH 1, $E_p = +0.7$ V, is due to the oxidation of the phenolic group. The second wave starting at pH 4, $E_p = +1.1$ V, corresponds to the oxidation of the tertiary amine group. It was found that the values of E_p for both peaks shifted to more negative potentials with increasing pH, (Fig. 2b), corresponding to a mechanism involving the same number of electrons and protons [23]. These results are in

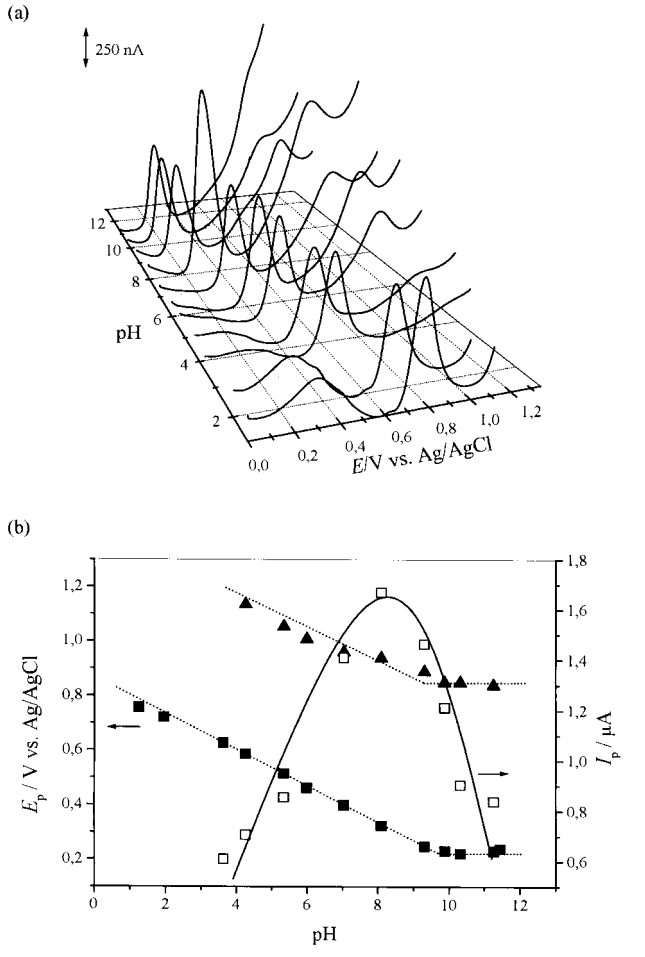


Fig. 2. a) 3D plot and b) plots of E_p (filled symbols) and I_p (open symbol) vs. pH from differential pulse voltammograms of 100 µM morphine in different buffer electrolytes as function of pH. Scan rate 5 mV s^{-1}.

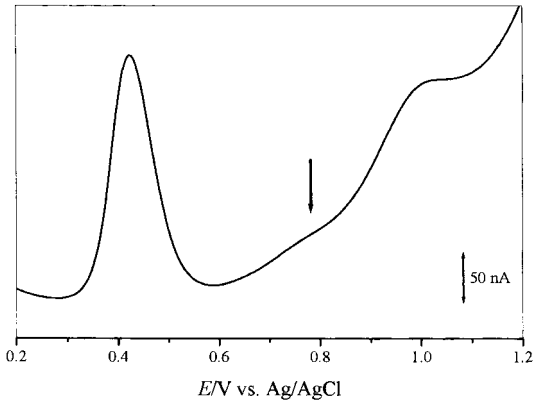


Fig. 3. Differential pulse voltammogram of a 100 µM solutions of morphine in pH 7 phosphate buffer. Scan rate 5 mV s^{-1}.

agreement with the data found in the literature [5]. However, a less obvious peak is also referred to between the first and the second peak; it was suggested to be due to further oxidation of pseudomorphine, the product resulting from oxidation of the phenolic group [5]. At first sight, from the results obtained in this work, it was not possible to observe the presence of this peak. However, expanding the current scale of the voltammograms obtained between pH 5 to 7 a small shoulder, indicated by the arrow, at approximately +0.8 V is observable (Fig. 3).

To clarify the occurrence of this peak, to prove that the groups involved in the oxidation are the phenolic and tertiary amine and in order to establish the oxidative pathways of morphine a comprehensive electrochemical study of several metabolites and derivatives was carried out. The electrochemical behavior of pseudomorphine, morphine *N*-oxide, normorphine, dihydromorphine and 2-(*N*,*N*-dimethylaminomethyl) morphine, was studied at a glassy carbon working electrode using differential pulse voltammetry over the pH range 1.2 to 12.2 (Fig. 4).

Considering the voltammetric behavior and molecular structure of normorphine and particularly of morphine *N*-oxide (Figs. 1, 4c and 4d) it can be readily concluded that the second anodic wave that starts at $E_p = +1.1$ V for morphine must correspond to the oxidation of the tertiary amine group, i.e., the non-existence of this wave for these compounds is related with the absence of a tertiary amine group in their structure. Since the oxidation of a tertiary amine gives rise to a secondary amine and an aldehyde [24] it can be deduced that one of the main oxidative products of morphine is normorphine (Scheme 1). Particular attention should be given to the second peak observed for normorphine (Fig. 4d). The appearance of this wave is related with the oxidation of the secondary amine group present in the molecular structure. Although this oxidative pattern is already referred to in the literature for secondary amines [25, 26], further evidence was obtained by studying a closely structurally related compound, norheroin (data not shown). The results obtained clearly demonstrate that the second peak observed for normorphine is indeed connected with the oxidation of the secondary amine group.

The assignment of the first peak for morphine at pH 1, $E_p = +0.7$ V, to the oxidation of the phenolic group could be easily proved. The oxidation of codeine or ethylmorphine which have methoxy and ethoxy groups, respectively, instead of a phenolic group in the 3-position, was studied and the first peak was obtained only at potentials higher than +1.0 V (data not shown). The oxidation of the phenolic group leads to the formation of pseudomorphine as the main product, as already suggested [3, 5] (Scheme 1). This product, obtained from oxidation of the phenolic group, is, according to the literature [5], responsible for the poorly defined peak occurring at +0.8 V for morphine, since the structure of pseudomorphine (Fig. 1b) possesses two phenolic groups that could be susceptible to further oxidation.

To verify this assumption the oxidative behavior of pseudomorphine was analyzed. As seen in Fig. 4b there are two well-defined peaks occurring at the same potential as for morphine (Fig. 4a). Moreover, at some pHs, it is also possible to observe a third poorly-defined peak at $E_p =$ +0.8 V. From these results it can be concluded that the

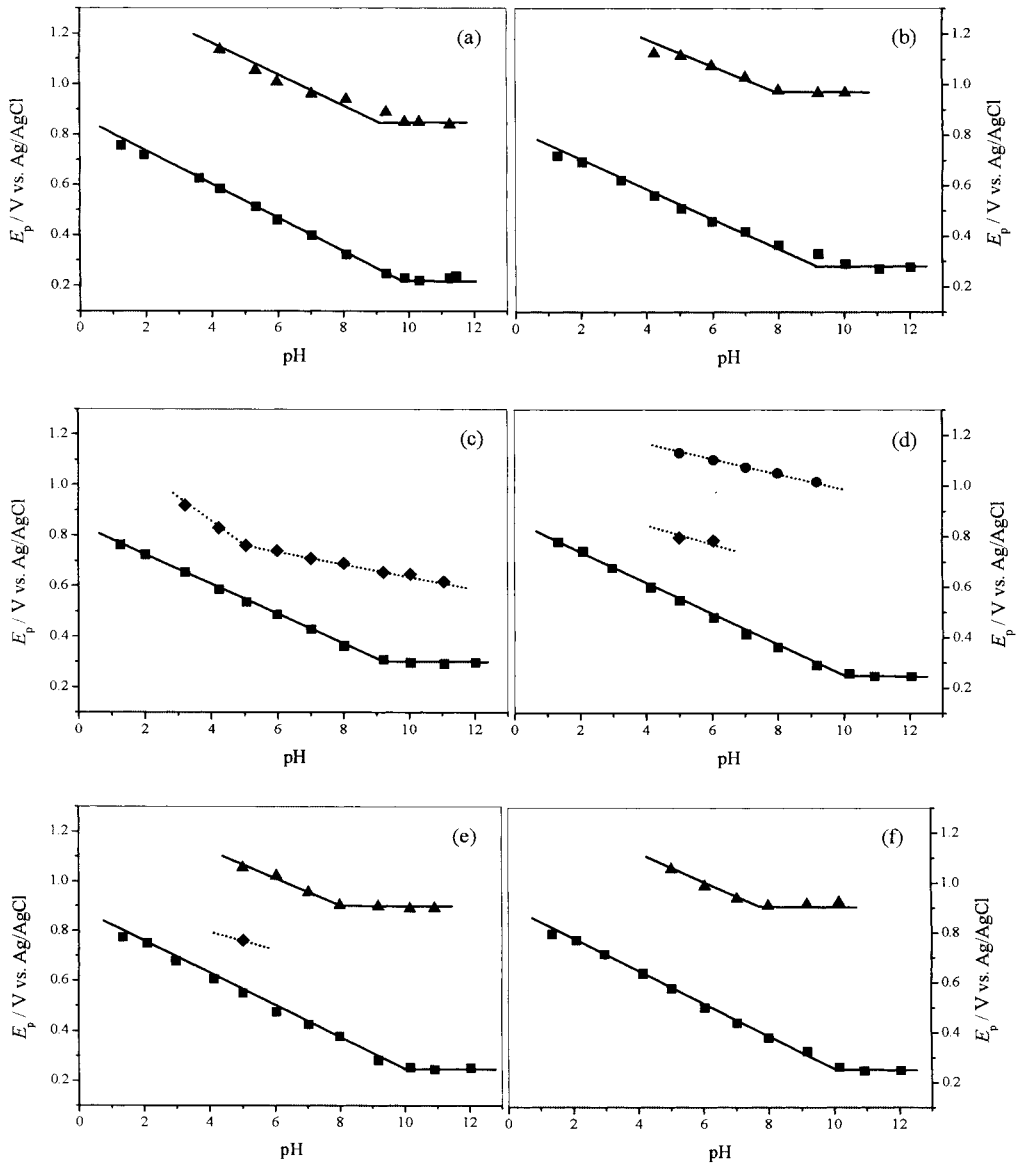
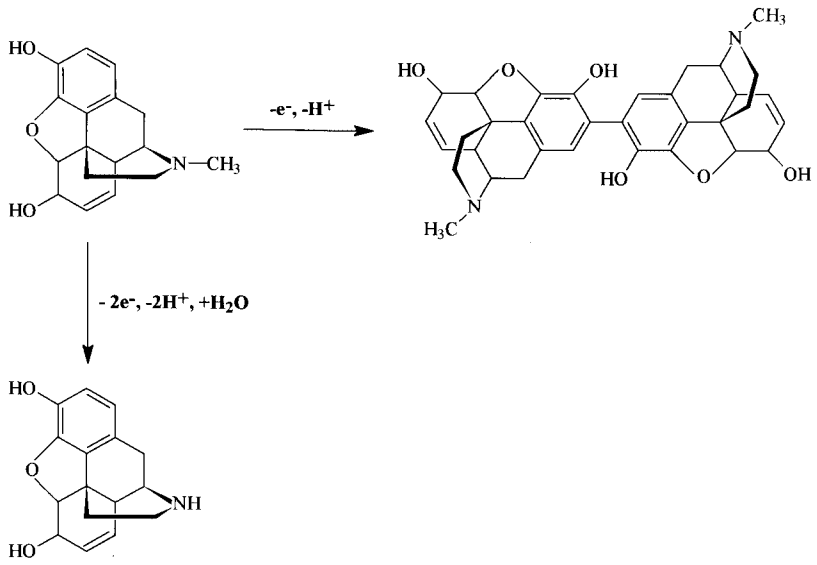


Fig. 4. Plots of E_p vs. pH from differential pulse voltammograms of 100 μM solutions of a) morphine, b) pseudomorphine, c) morphine *N*-oxide, d) normorphine, e) dihydromorphine and f) 2-(*N*,*N*-dimethylaminomethyl)morphine in different buffer electrolytes. Scan rate 5 mV s^{-1}. (—) Slope 59.2 mV/pH unit.

presence of two phenolic groups in the pseudomorphine molecule makes possible its further oxidation but, as seen, the oxidation occurs at the same potential as morphine. Another important deduction is that the peak current for pseudomorphine for all pHs studied is approximately double that obtained for morphine (Fig. 5). This is expected given the presence of two symmetric phenolic groups in the pseudomorphine molecule. Thus, if the product of oxidation of the phenolic group present in morphine, was pseudomorphine, which then underwent further oxidation the peak obtained would not appear at the cited potential +0.8 V.

This occurrence of the peak at +0.8 V assumes more importance considering that it appears very well-defined at several pHs in the voltammograms of morphine *N*-oxide and normorphine (Figs. 4c, 4d and 6). The oxidation of aliphatic alcohols usually occurs at high potentials. However, the presence of a double bond in morphine in the neighborhood of the hydroxy group at the 6-position, that also appears in morphine *N*-oxide and normorphine (Fig. 1a, 1c and 1d), will influence the mechanism. The fact that it is stated in the literature that a metabolite resulting from the oxidation of this group (morphinone) occurs in vivo [27], led us to study the electrochemical behavior of dihydromorphine (Fig. 1e). As seen in Figure 4e, this compound has an identical oxidation profile to that of morphine and so it is unlikely that the hydroxy group in the 6-position is responsible for the unidentified peak.

In order to verify if the formation of a dimer, different from pseudomorphine, could be responsible for the appearance of this peak, a derivative of morphine blocked at the 2-



Scheme 1.

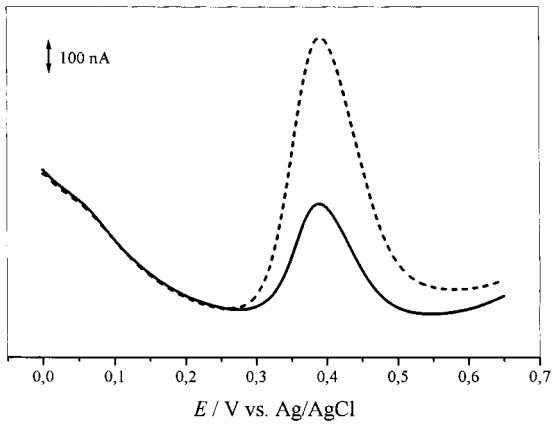


Fig. 5. Differential pulse voltammograms in pH 7 phosphate buffer of 100 μM solutions: (—) morphine and (–) pseudomorphine. Scan rate 5 mV s^{-1}.

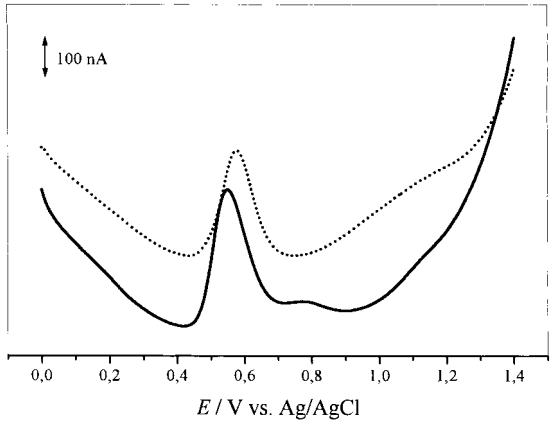


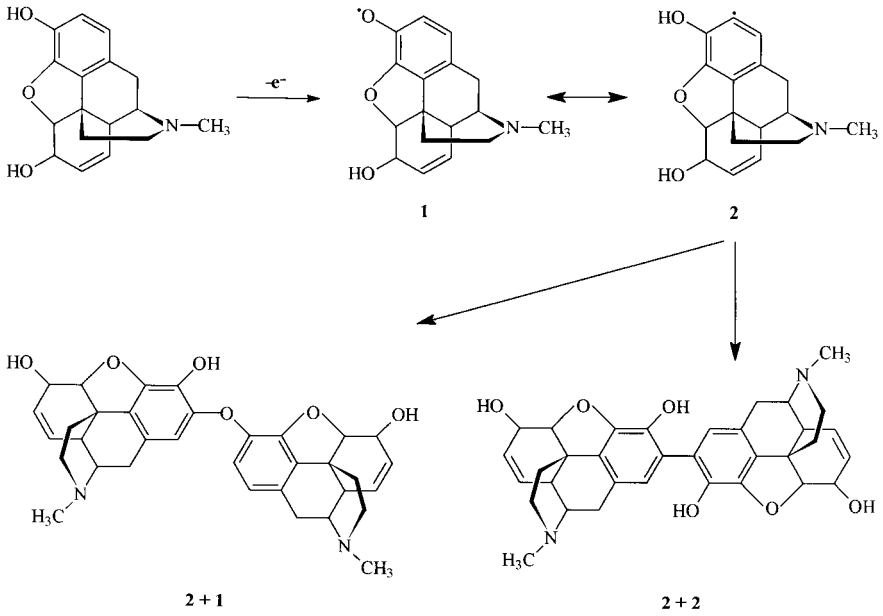
Fig. 6. Differential pulse voltammograms in pH 5 acetate buffer of 100 μM solutions: (–) morphine *N*-oxide and (·····) 2-(*N*,*N*-dimethylaminomethyl) morphine. Scan rate 5 mV s^{-1}.

position, 2-(*N*,*N*-dimethylaminomethyl) morphine (Fig. 1f), was synthesized and studied voltammetrically (Fig. 4f). Figure 6 shows the behavior of morphine N-oxide and 2-(*N*,*N*-dimethylaminomethyl) morphine at pH 5, the pH at which the peak at +0.8 V was best defined for morphine. The results obtained show that this peak is absent in 2-(*N*,*N*-dimethylaminomethyl) morphine at all pHs and therefore, an unrestrained 2-position is necessary for appearance of this peak.

Thus, considering the results discussed previously, the peak at +0.8 V should result from further oxidation of a dimer (Scheme 2), other than pseudomorphine, arising from the oxidation of the phenolic group present in morphine. The formation of a second, different dimer, other than pseudomorphine, by partial oxidation of morphine has already been reported in the literature [28].

The occurrence of another dimeric form is also proposed during the degradation of morphine and consequent production of pseudomorphine by direct analogy to what is found for the oxidation of *p*-cresol [2]. Considering the oxidation mechanism for phenol the occurrence of this second dimer can be explained. It is well-known that the oxidation of a phenolic group gives rise to a semiquinone that exists in equilibrium with the corresponding quinone (Scheme 2). According to the literature, it is this quinone that undergoes coupling with protonated or free base morphine resulting in the formation of the dimer pseudomorphine [2]. Nevertheless, the existence of the semiquinone in the equilibrium cannot be neglected leading to the formation of dimeric forms such as an ether (Scheme 2).

In this way, it is reasonable to suppose that the unidentified peak, at +0.8 V, obtained for the oxidation of morphine is a consequence of further oxidation of this dimer formed jointly with pseudomorphine by oxidation of the phenolic group. A similar dimer was recently prepared by oxidation of 2-hydroxymethylmorphine making this hypothesis even



Scheme 2.

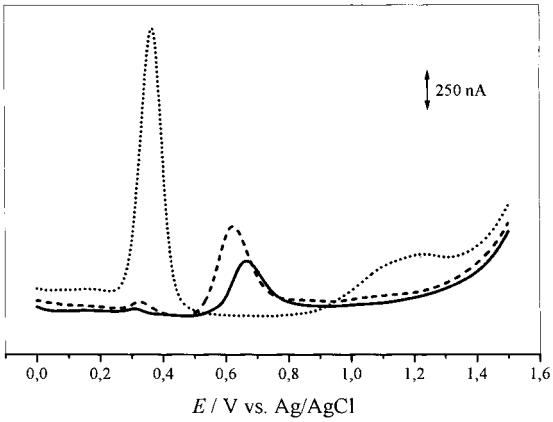


Fig. 7. Differential pulse voltammograms in pH 3 acetate buffer of 100 μM solutions: (—) morphine, (····) apomorphine and (- - - -) pseudomorphine. Scan rate 5 mV s^{-1}.

more likely [29]. This dimer and the possibility of further oxidation leading to polymers could also be an explanation for the yellowish to brown discoloration observed in morphine solutions during storage.

Apomorphine has been proposed as a degradation product of morphine [30] and since this constitutes a subject of some controversy [31] some studies were done with a view to contributing to clarify this matter. Nevertheless, the formation of apomorphine from morphine appears to require heating up to 60–65 °C in concentrated HCl for 2–3 h [31, 32]. The voltammetric results obtained in pH 3 acetate buffer for morphine and pseudomorphine (Fig. 7) suggests that apomorphine can also be a minor product formed as a result of the occurrence of a side reaction. In fact, comparing the peak appearing at this pH, for both compounds, at approximately $E_p = +0.3$ V with the voltammetric behavior of apomorphine at the same pH some similarities are encountered (Fig. 7). Considering that the acidic rearrangement of morphine to apomorphine is already well described [32] and in order to verify if the amplitude of this peak is time-dependent a study was carried out in pH 3 acetate buffer for morphine. The results obtained are not conclusive since an increase in peak current occurred during the first 5 h but there was a sudden decrease after 24 hours. This behavior could in part be justified by the oxidative profile of apomorphine since in aqueous solutions degradation easily occurs [33]. An attempt to prove apomorphine formation by cyclic voltammetry was also unsuccessful since this technique does not have sufficient sensitivity and it was not possible to detect the occurrence of this peak for morphine. More studies need to be carried out in order to clarify and justify the results obtained.

4. Conclusions

The electrochemical oxidative behavior of morphine in aqueous solution was studied and was found to be complex and pH-dependent. To elucidate and clarify the voltammetric waves obtained several metabolites and derivatives, pseudomorphine, morphine *N*-oxide, normorphine, dihydromorphine and 2-(*N*,*N*-dimethylaminomethyl)morphine, were synthesized and studied.

Two well-defined anodic peaks were found for morphine oxidation that were related, to the oxidation of phenol and tertiary amine groups, after a detailed study and comparison with the oxidative profile of several congener compounds. Moreover, the poorly defined peak seen for morphine oxidation is clearly visible for normorphine and morphine *N*-oxide suggesting that it is related with further oxidation of a dimer different from pseudomorphine. This conclusion

could be very important for interpreting discoloration phenomena of morphine solutions besides contributing to understand oxidative metabolic pathways and biological interactions of morphine.

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6. Supporting Information Available

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

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