Electrochemical oxidation of amphetamine-like drugs and application to electroanalysis of ecstasy in human serum

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A B S T R A C T

Amphetamine and amphetamine-like drugs are popular recreational drugs of abuse because they are powerful stimulants of the central nervous system. Due to a dramatic increase in the abuse of methylenedioxylated derivatives, individually and/or in a mixture, and to the incoherent and contradictory interpretation of the electrochemical data available on this subject, a comprehensive study of the redox properties of amphetamine-like drugs was accomplished. The oxidative behaviour of amphetamine (A), methamphetamine (MA), methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) was studied in different buffer systems by cyclic, differential pulse and square-wave voltammetry using a glassy carbon electrode. A quantitative electroanalytical method was developed and successfully applied to the determination of MDMA in seized samples and in human serum. Validation parameters, such as sensitivity, precision and accuracy, were evaluated. The results found using the developed electroanalytical methodology enabled to gather some information about the content and amount of MDMA present in ecstasy tablets found in Portugal. Moreover, the data found in this study outlook the possibility of using the voltammetric methods to investigate the potential harmful effects of interaction between drugs such as MDMA and methamphetamine and other substances often used together in ecstasy tablets.

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1. Introduction

Recreational drugs have always played a part in the human society. Some of the psychotropic drugs have been used since pre-history, but in many countries are proscribed and are consequently subject to clandestine synthesis and illegal traffic world-wide. Amphetamine-type drugs are abused by about 34 million people in the world [1]. In Portugal, amphetamine-type drugs, account for a greater part of illicit drugs seizure and consumption [2]. Amphetamine and amphetamine derivatives have become popular recreational drugs of abuse because they are powerful stimulants of the central nervous system; they increase self-confidence and wakefulness and improve physical performance. In particular, a dramatic increase in the abuse and recreational use of methylenedioxylated amphetamine derivatives has been detected in many countries, especially among young people. The most important substance in this group is 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) which is classified as illicit substance in most countries. The recreational use of MDMA is prevalent, mainly among youth, despite warnings of irreversible damage to the central nervous system [3]. Furthermore, MDMA consumption produces a variety of systemic and organ-specific adverse effects [4]. Consequently, MDMA abuse has the potential to give rise to a major public health problem. Ecstasy usually is sold in the form of pills or tablets with distinctive patterns and shapes. The amount of MDMA varies enormously from tablet to tablet, but commonly a typical dose is 100 mg MDMA per tablet. However, the composition could differ considerably. In Portugal little is known about the content and amount of MDMA present in ecstasy tablets.

The growing manufacturer’s desires to maintain the supply of ecstasy tablets needed to meet consumer demand may have played a part in the increase of ecstasy tablet contamination that occurred in the last years. The percent of pure ecstasy tablets decreased over time, largely due to an increase in tablets sold with mixtures of MDMA along with other substances. The overwhelming presence of non-MDMA substances in ecstasy tablets in some countries is alarming and has important public health implications. It is possible that non-MDMA substances in ecstasy tablets, alone or in combination with MDMA may be partly responsible for the discrepant research findings regarding neurotoxicity-related memory problems [5].
Therefore, there is an increasing interest in the study and development of a rapid, selective and sensitive method for the identification and quantification of MDMA in seizure samples and biological fluids, so that an effective control of these drugs can be done.

The identification and quantification of amphetamines in biological samples have been described using a variety of methodologies. Chromatographic techniques, such as high performance liquid chromatography (HPLC) or gas chromatography (GC), and capillary electrophoresis are the most commonly used methodologies for the analysis of ecstasy [6–9].

Electroanalytical techniques have become indispensable tools in modern analytical chemistry, and electrochemical methods have also been used for determining amphetamine-type drugs [10–14]. Despite these applications, the understanding of the electrochemical oxidation mechanism of amphetamine-like compounds is not clarified. In fact, after the report, fifteen years ago, about the voltammetric behaviour of 3,4-methylenedioxyamphetamine (MDA) [15] no other study on amphetamine’s electrochemical properties has recently been done. This lack of information led sometimes to incoherent interpretation of the data found and to contradictory results [16,17].

In order to clarify the electrochemical oxidation of amphetamine-like drugs, a comprehensive voltammetric study of amphetamine (A), methamphetamine (MA), methylenedioxyamphetamine (MDA) and methylenedioxyamphetamine (MDMA) (Fig. 1) was undertaken, and a voltammetric method was developed for the quantification of MDMA in seizure samples and in human blood serum. As a final goal, this study intends to gather some information about the content and amount of MDMA present in ecstasy tablets found in Portugal.

2. Experimental

2.1. Apparatus

Voltammetric experiments were performed using an Autolab PGSTAT 12 potentiostat/galvanostat (EcoChemie, Netherlands) associated to an one-compartment glass electrochemical cell equipped with a three-electrode system consisting of a glassy carbon working electrode (GCE) (d = 2 mm), a platinum wire counter electrode and an Ag/AgCl saturated KCl reference electrode. The glassy carbon working electrode was polished manually with aqueous slurry of alumina powder (BDH) on a microcloth pad and rinsed with water before use. The electrode was then cycled in the potential region of the voltammetric measurements until a reproducible response was obtained. All measurements were made at room temperature.

The optimum instrumental parameters used in the square-wave (SW) voltammetry quantification were chosen studying the variation of the peak current (Ip) with the SW frequency (f), pulse amplitude (Ep) and ionic strength (I). The system was optimised, particularly in respect of maximum peak current and reproducibility.

A Crison pH-meter with glass electrode was used for the pH measurements (Crison, Spain). The HPLC experiments [18] were performed using a HPLC/DAD, Shimadzu instrument (pumps model LC-20AD, Tokyo, Japan), equipped with a commercially prepacked Tracer Excel 120 ODS-B analytical column (250 mm × 4.0 mm, 5 μm, Teknokroma, Barcelona, Spain) and UV detection (SPD-M20A) at the maximum wavelength determined by the analysis of the UV spectrum (210 nm). The mobile-phase consisted of methanol/ammonium acetate buffer 50 mM (40:60) containing 0.1% triethylamine, pH 3.9, at a flow rate of 0.8 mL/min at room temperature. The chromatographic data was processed in a Siemens computer, fitted with LabSolutions software (Shimadzu, Japan).

2.2. Chemicals and standards

Amphetamine sulfate and methamphetamine hydrochloride were supplied by Sigma-Aldrich Química (Sintra, Portugal). MDMA and MDA were synthesized as described elsewhere [16]. Analytical grade reagents purchased from Merck (Darmstadt, Germany) were used without additional purification. Seized “Ecstasy” tablets were provided by Policia Judiciária (Lisbon, Portugal).

Deionised water (conductivity < 0.1 μS cm−1) was used throughout the experiments. Buffer solutions, used as supporting electrolytes, were 0.2 M ionic strength and the pH range of 1.2–12.2.

HPLC-grade methanol was obtained from Merck. Ammonium acetate buffer 50 mM was prepared in deionised water and the pH was adjusted to pH 3.9. Prior to use, the solvents were filtered through a 0.45-μm filter.

Standard stock solutions of A, MA, MDA and MDMA (2.5 mM) were prepared in deionised water. In a typical run, 10 mL of supporting electrolyte was transferred to the electrochemical cell and the required volume of the standard stock solution was added to the electrolyte, in order to get a final concentration of 0.1 mM. For calibration curves, standard solutions were prepared in the voltammetric cell adding accurate volumes of the stock standard solution of MDMA to the selected phosphate pH 7.3 supporting electrolyte in order to obtain concentrations between 8 and 45 μM. The calibration curve for SW voltammetry was constructed by plotting the peak current against the MDMA concentrations (8.7, 17.4, 26.1, 34.7 and 43.4 μM). The limit of quantification (LOQ) and the limit of detection (LOD) were calculated according to USP guidelines [19]. A S/N ratio of ten and three was used respectively (LOD = 3 × S.D × (sensitivity)−1, LOQ = 10 × S.D × (sensitivity)−1). The method precision was checked at different days, within day (n = 5) and between days (n = 5) for three different concentrations. The accuracy of the proposed method was determined by comparing the results with those obtained using a previously published HPLC method [18]. The precision and accuracy of the analytical method was also described by the use of relative errors (Bias%).

2.3. Analysis of seized ecstasy tablets

A total of twelve tablets containing MDMA were analysed in this study. For MDMA determination each tablet was weighed and ground to a homogeneous fine powder in a mortar. An accurately weighed portion equivalent to a stock solution of a concentration about 2.5 mM was transferred to a volumetric flask and dissolved with deionised water. The mixture was sonicated for 5 min to attain complete dissolution and filtered to remove any remaining insoluble matter. Working solutions of the seized ecstasy tablets were prepared exactly as the standard solutions.

2.4. Analysis of spiked serum samples

Human serum samples were collected from healthy volunteers and stored frozen until the assay. An aliquot volume of sample was
fortified with MDMA and diluted in deionised water to achieve the final concentration of 2.5 mM. The mixture was treated with 0.4 mL of methanol as serum denaturating and precipitating agent, and then the volume was completed to 1.5 mL with the same serum denatured sample. The tubes were vortexed for 5 min and then centrifuged for 10 min at 4000 rpm for removing of protein residues. The supernatant was taken carefully. Appropriate volumes of this solution were added to phosphate pH 7.3 supporting electrolyte and the voltammograms were then recorded.

The concentration of MDMA in the human serum samples varied in the range of 12 to 45 μM. For the recovery studies the amount of MDMA in spiked human serum samples was calculated from the related calibration equation.

Methanol and acetonitrile, in different amounts, were tested as serum protein precipitating agents. The best results were obtained using 0.4 mL of methanol.

3. Results and discussion

The alarming increase in the recreational use of amphetamine-like drugs, in particular MDMA, and the multitude of adverse effects resulting from its misuse require a complete understanding of the pharmacological and toxicological profile of these amphetamine derivatives. To date, there is a general consensus that MDMA metabolism, resulting in the formation of highly redox active metabolites, is required for the expression of MDMA-induced toxicity [20,21].

To gain insight into the possible formation of these metabolites and to provide additional information about the mechanism by which they could exert their toxic effects, the electrochemical behaviour of A, MA, MDA and MDMA (Fig. 1) was studied over the pH interval 1.2 to 12.2, at a glassy carbon electrode using different voltammetric techniques.

3.1. Electrochemical oxidation

Differential pulse (DP) voltammetry of A showed, as expected, that it is not electroactive over the entire pH range studied, since A possesses in its molecular structure only a single functional group, an aliphatic primary amine. Primary amines usually oxidise at potentials higher than those allowed by the potential window of the glassy carbon electrodes.

For MA, a single anodic wave can be observed above pH 9, $E_p = +0.92$ V (Fig. 2A), corresponding to the oxidation of the secondary amine present in the MA molecule. The slope of the dotted line (Fig. 2B), ca. 60 mV per pH unit, shows that the mechanism of this oxidation process in aqueous media involves the same number of electrons and protons. The appearance of a peak at this potential has also been described in the literature for other secondary aliphatic amines [22–24].

The first step in the anodic oxidation of aliphatic amines in aqueous solution is considered to be the abstraction of an electron from the lone-pair of electrons on the amino-nitrogen. In fact the MA secondary amine group is more easily oxidised in basic solution than...
in acidic solution, and this data correlates well with the $pK_a = 10.1$ of the amine function of MA [25]. Cyclic voltammograms obtained showed the irreversible oxidation of this compound, as one well-defined anodic peak and no reduction peak was observed.

A single anodic peak over the entire pH range examined, $E_p = +1.17$ V at pH 2 (Fig. 3A), was observed for MDA, corresponding to the removal of one electron from the aromatic nucleus present in the molecule and subsequent formation of a radical cation. The anodic peak potential was shifted to more negative values on increasing the solution pH, Fig. 3B. The $E_p$–pH plot shows that the electrode process is pH-dependent, the slope of the dotted line (Fig. 3B), ca. 30 mV per pH unit, shows that the mechanism of this oxidation process in aqueous media involves a number of electrons that is double the number of protons.

Cyclic voltammograms recorded also showed one irreversible anodic peak. This result indicates that a very fast subsequent chemical reaction of the radical cation generated on the anodic oxidation takes place, so that the reduction of the radical cation will not occur in the time scale of the experiment. This is well documented for related compounds [15,26–28]. However, at high scan rates this oxidation wave can exhibit partial reversibility, as was demonstrated using low temperature ESR spectroscopy [29].

Excluding the peak observed at $+0.35$ V for pH 3 and 4 that is related with the GCE background current, the DP voltammetry of MDMA showed the first anodic peak, P1 at $E_p = +1.18$ V, starting at pH 2 (Fig. 4A), corresponding to an oxidation on the aromatic nucleus of the molecule leading to the formation of a radical cation (Scheme 1). The $E_p$–pH plot (Fig. 4B) shows that peak P1 electrode process is pH-dependent over the whole pH range; $E_p$ decreases linearly with increasing pH. The slope of the dotted line, ca. 30 mV per pH unit, shows that the mechanism of this oxidation process, in aqueous media involve a number of electrons that is double the number of protons.

The second anodic peak, P2 at $E_p = +1.31$ V starts appearing at pH 4 (Fig. 4A), and is related to the oxidation of a species formed by dimerization of the initial radical cation (Scheme 1). The current observed is in good agreement with the formation of a dimeric product [26,27]. The slope of the dotted line, ca. 30 mV per pH unit, shows that the mechanism of this oxidation process, P2, in aqueous media involves a number of electrons that is double the number of protons.

Above pH 9 another anodic peak, P3 at $E_p = +0.86$ V at pH 9 (Fig. 4A), corresponding to the oxidation of the secondary amine present in the MDMA molecule (Scheme 1) is observed. The appearance of this peak is related with the acid–base properties of MDMA. In fact, at this pH deprotonation of the secondary amine group occurs and, as a result, an oxidative process can take place due to the existence of an electron lone-pair. This data not only correlates well with the $E_p$–pH behaviour observed for the other anodic waves but is also consistent with the voltammetric profile of methamphetamine previously described. The values of $E_p$ for peak P3 are pH-dependent, (Fig. 4B), and the slope of the dotted line, ca. 60 mV per pH unit, shows that the mechanism of P3 oxidation process in aqueous media involves the same number of electrons and protons (Scheme 1).
Based on the data obtained for all the amphetamine compounds studied, an oxidation mechanism is proposed for MDMA (Fig. 5, Scheme 1). In fact, the only structural difference between MDMA and MDA lies in the amine group. MDMA has a secondary and MDA a primary amine group. Thus, the peak P3 observed for MDMA starting at pH 9 is related with the oxidation of the secondary amine group. This pattern is consistent with the voltammetric behaviour described, for A and MA, molecules containing also primary and secondary amines, respectively.

The appearance of the peak P2 at pH 4, for MDMA is clarified below. It was expected that a similar peak would be present for MDA voltammetric profile since this oxidative signal is attributed to oxidation of a species ensuing from the formation and dimerization of the initial radical cation, occurring both in MDMA and MDA. The use of controlled potential electrolysis (CPE) was considered to clarify this process; nevertheless it was apparent from the literature that no significant conclusion would result from this study. Preparative electrolysis at a fixed potential was done for similar compounds in order to understand the mechanism of this type of oxidations [26,27]. The oxidation potentials of the products obtained were characterized at the end of the electrolysis and none of them corresponded to the potential observed for P2. It was concluded that the dimeric species responsible for this oxidation peak was an intermediate formed in route to the final products [26,27].

To clarify the process occurring at peak P2 in MDMA the following was investigated. It seemed reasonable that the alkyllic chain, containing the amine group, attached to the aromatic nucleus would have some influence on the stabilization of the initial radical cation. If that occurs then this could be the main reason for the unexpected disappearance of the wave in MDA. In order to test this hypothesis, the voltammetric behaviour of 1,2-(methylenedioxy) benzene and 3,4-(methylenedioxy)toluene was studied at pH 7, and the same pattern was observed for these two model compounds (Fig. 6). The introduction of a methyl group as a substituent in the aromatic nucleus seems to affect significantly the stabilization of the radical cation formed since it produced an anodic peak potential shift to more negative values. This lower potential value results from the increased stabilization of the radical cation originated by the electron-donating effect of the methyl group. This explains the apparent difference in the peak potentials between MDMA and MDA.

In view to an electroanalytical application, the influence of the pH on the MDMA peak current at a glassy carbon electrode was also investigated, using DP voltammetry. The plot of \(I_p\) vs. pH indicates that the peak current reaches a maximum for pH 7 (Fig. 4B). The experimental results also showed that the DP voltammograms are better defined in pH 7 phosphate buffer as compared to Britton-Robinson buffer. Consequently, this buffer was chosen as the best supporting electrolyte and was used throughout the electroanalytical study.

Cyclic voltammograms were also recorded at different sweep rates at pH 7. Two well-defined anodic peaks, P1 and P2, were observed for MDMA (Fig. 7). No cathodic peak was observed for the scan rates investigated (from 10 mV s\(^{-1}\) to 500 mV s\(^{-1}\)).

The effect of the potential scan rate (10–500 mV s\(^{-1}\)) on the peak current of MDMA was evaluated, according to the equation \(I_p = A\sqrt{v}\). The x values of 0.5 and 1.0 are expected for diffusion and adsorption controlled process [30]. A plot of the logarithm of peak current versus logarithm of scan rate yielded a straight line of slope 0.52, indicating diffusion controlled process [30]. The peak potential was shifted to more positive values on increasing the scan rate, which confirms the irreversible nature of the oxidation process.

### 3.2. Electroanalytical applications

Based on the voltammetric behaviour of MDMA, a quantitative electroanalytical method was developed to determine the drug content in different types of samples. To select the best electrochemical method for this purpose, the anodic peaks obtained by DP and SW voltammetry were compared. SW voltammetry was found to be the faster method, giving the best ratio of peak-to-background current and providing sharper and better defined peaks, leading to an enhanced resolution.

The optimum instrumental parameters used in the SW voltammetry quantification were chosen studying the variation of the peak current (\(I_p\)) with the SW frequency (\(f\)), pulse amplitude (\(E_s\)) and ionic strength (\(l\)). The system was optimised, particularly in respect to maximum peak current and reproducibility. Optimization of the SW voltammetry parameters regarding peak definition and current was accomplished using \(f = 100\) Hz, \(E_s = 50\) mV and \(l = 0.2\) M. Under the described experimental parameters, SW voltammograms recorded with increasing amounts of MDMA (8 to 45 µM) showed that the peak currents increased linearly with increasing concentration. Electroanalytical data for the calibration: limit of detection (LOD), limit of quantification (LOQ), obtained after five runs, and precision of the method, evaluated by repeatedly (\(n = 5\)) measuring MDMA, at three levels of concentration (12, 30 and 45 µM) within a day and over five consecutive days, are summarized in Table 1.

### 3.3. Electroanalysis of seized ecstasy tablets

Considering the lack of information regarding the amount of MDMA present in seized ecstasy tablets in Portugal, and based on the developed
Recent ecstasy tablet purity surveys in Europe suggested that MDMA purity rates are around 90% [31,32]. In fact, the majority of the seized or collected tablets contain only MDMA with no other active substance added and only excipients, mainly sugars: glucose, lactose, sorbitol, saccharose, and fatty acids. Occasionally found non-MDMA active substances were usually other amphetamine-like molecules: MDEA, A, MDA, and MA. The blended psychoactive substance most frequently found in the tablets containing MDMA is caffeine [31]. None of these active substances were detected by the SW voltammetry and HPLC methodologies used in the present study.

To confirm the accuracy of the proposed method and to evaluate the possible interaction with excipients, recovery experiments were carried out after the addition of known amounts of the pure drug to MDMA seized tablets. The results showed that the excipients are electrochemically inactive, have no interference effect on the analysis, Table 2, and both methodologies applied had a comparable precision and accuracy.

### 3.4. Determination of MDMA in serum samples

In order to apply the proposed method to biological samples, the quantification of MDMA in human serum was tested. Calibration equation parameters and validation data are summarized in Table 1. Serum samples were spiked with MDMA in order to achieve final concentrations of 15, 30 and 45 µM. SW voltammograms obtained for the determination of MDMA in human serum samples are in Fig. 8. The recoveries obtained were good and are shown in Table 3.

### 4. Conclusions

The voltammetric behaviour of several amphetamine-like drugs, A, MA, MDA and MDMA was investigated. The analysis performed showed that, with the exception of A, all the amphetamines studied are electroactive and their oxidation mechanism is related to an oxidation process taking place on the aromatic nucleus and/or on the secondary amine group present in the molecules. The data found also contributed to clarify the oxidative and metabolic profile of MDMA in human serum samples.
amphetamine-like drugs. A SW voltammetry methodology was developed for the determination of MDMA in seized tablet samples and biological samples. The results showed that the electroanalytical method is sensitive and does not lead to statistically significant differences when compared to the established HPLC methodology. Despite the limited size sample used, it was found that the ecstasy tablets analysed have MDMA purity rates of ~100% and the dosage levels of MDMA in seized tablets were ~ 120 mg.

Although most ecstasy users are aware of the drug’s associated risks, they may still be unaware of the risks they face from adulterated tablets. The reported results in this study outlook the possibility of using the voltammetric methods to investigate the potential harmful effects of interaction between drugs such as MDMA and methamphetamine and other substances often used together in ecstasy tablets.

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