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Development of an electroanalytical method for the quantification of aminopyrine in seized cocaine samples

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ABSTRACT

We report the development of a simple and accurate analytical method to detect aminopyrine in seized cocaine samples using a platinum electrode. The quantification of aminopyrine was performed using square wave voltammetry (SWV), where the peak current response was found to increase linearly with aminopyrine concentration over the range 100–1000 μmol L^{-1}. The repeatability of the electrode response was determined to be 2.4% (n = 15), and the detection limit of the proposed method was estimated to be 22 μmol L^{-1} (3σ/slope). The accuracy of the proposed method was evaluated comparing the measured aminopyrine concentration in seized cocaine sample to the value obtained by gas chromatography coupled to flame ionization detector (GC-FID). An addition and recovery protocol in seized cocaine samples was also performed.

1. Introduction

Aminopyrine or 4-dimethylamino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one has been widely used as an analgesic and antipyretic drug and as a model substrate for *in vitro* and *in vivo* investigations of drug metabolism [1–3]. Aminopyrine can cause agranulocytosis and bone marrow suppression, decrease the amount of white blood cells, and form nitrosamines, which are carcinogenic substances [1,2]. For these reasons, this drug has been withdrawn from the market in some countries [4].

In the literature, aminopyrine can be quantified by many different analytical techniques such as electroanalysis [1,5], high-performance liquid chromatography (HPLC) with ultra-violet detection (UV/Vis) [6], capillary electrophoresis with electrochemical detection [7], piezoelectric [8], and mass spectrometry [2]. Most of these techniques require labour intensive or well-trained professionals and involve several analytical steps and high-cost equipment. In comparison with these techniques, electroanalytical methods offer many advantages, such as simplification of the analytical procedures, time and cost savings, and ease of miniaturization for transporting the system, which could be a highlight considering the application of this method for forensic measurements in field.

Aminopyrine is commonly found in seized cocaine samples [9]. Pharmaceutical formulations are used as adulterants of drugs of abuse, such as cocaine, to mimic the physical proprieties and pharmacological effects of the drug or to mask the dilutions made by traffickers to increase the volume of drugs and thus, their profits. Additional substances may be added to bulk, dilute, complement, or enhance the effects of the drugs. Others are present unintentionally, being added as the result of manufacturing, production, or storage processes [10].

The identification and quantitation of cocaine and its adulterants, and additional substances, are important not only from a toxicological and clinical perspective, but also for forensic analysis to determine trends on drug production and changes in cocaine traffic dynamics (geographical origin). The analysis of different seizure samples can also be useful for police intelligence purposes once the identification of adulteration patterns in drug of abuse samples made by traffickers in clandestine laboratories is important to track drug origin, as well as, international traffic. Therefore, detection of these adulterants is like a "fingerprint" of the seizure samples and this information can help in anti-trafficking policy efforts [11–13].

To the best of our knowledge, no electrochemical method using a bare platinum electrode has been used to quantify aminopyrine, and no methodology for the analysis of this compound for forensic applications was found in the literature. However, there is only one report related to quantification of aminopyrine in pharmaceutical formulations using a modified glassy carbon electrode [1]. This study used 3-aminopropyltriethoxysilane coated, Fe_3O_4 nanoparticles to modify the glassy carbon electrode. It is well known that nanoparticles can become electrochemically inactive during long measurements, and long exposition periods in field measurements, due to contact with air resulting in

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modifications to the surface and electroactive properties. This is a related drawback of the high reactivity of nanoparticles found in the literature [14,15]. Hence, this paper describes a simple, accurate, and fast method without chemical modification of the electrode surface for quantification of aminopyrine in cocaine samples seized by Sao Paulo State Police/Brazil. We believe that this study may be important and useful for forensic intelligence purposes as previously mention.

2. Experimental

2.1. Chemical and solutions

All chemicals were of analytical grade and used without additional purification. Solutions were obtained by dissolving the reagents in appropriate electrolytes. H_2SO_4, KH_2PO_4, K_2HPO_4, and NaOH were obtained from Merck (Darmstadt, Germany). Phenacetin, lidocaine, procaine, and benzocaine were obtained from Sigma-Aldrich (Steinheim, Germany). The aminopyrine was obtained from Alfa Aesar (Johnson Matthey Company). The seized cocaine samples were obtained from the Criminalistics Institute of São Paulo, SP, Brazil and were manually crushed and homogenized.

2.2. Electrodes and instrumentation

A μAUTOLABIII (Eco Chemie, The Netherlands) potentiostat with data acquisition software made available by the manufacturer (GPES 4.9.007 version) was used for the electrochemical measurements. All measurements were carried out in a three electrochemical cell using a homemade Ag/AgCl (saturated KCl) [16], platinum wire, and platinum rod as the reference, auxiliary, and working electrodes, respectively. Instead of the platinum electrode, different working electrodes (gold and glassy carbon) were tested in order to verify which material resulted in a better detectability for aminopyrine.

2.3. Electrochemical measurements

2.3.1. Aminopyrine quantification

The analytical aminopyrine solution and seized cocaine samples were diluted with 0.1 mol L^{-1} phosphate buffer (pH = 7.4). The working electrode was polished, using an alumina suspension (1 μm, Alfa Aesar, MA, USA) with a microcloth polishing pad, between each electrochemical measurement and washed thoroughly with deionised water in order to remove adsorbed species, and thus, obtain better reproducibility. For electrochemical aminopyrine analysis, square wave voltammetry was performed with the voltage potential ranging from 0.2 to 0.6 V. The optimum values for the step, amplitude, and frequency were 4 mV, 50 mV, and 120 Hz, respectively. The recovery test was performed by the addition of an amount of solid aminopyrine in the real seized cocaine samples to obtain a final concentration in solution of 200 μmol L^{-1}.

2.3.2. Rotating disk electrode experiments

Rotating disk electrochemical (RDE) experiments were carried out using an analytical rotator (AFMSRX, Pine Instrument Company) connected to the μAUTOLABIII potentiostat, recording current potential curves typically at a 20 mV s^{-1}. The platinum disk electrode has an outer diameter of 0.56 cm. The electrodes were polished using 0.3 μm alumina before using. A platinum wire and Ag/AgCl (saturated KCl) electrode were used as the counter and reference electrodes, respectively. During the rotating disk electrode experiments, the disk electrode potential was scanned between the limits −0.2 and 1.2 V.

2.3.3. Interference test

In order to evaluate the selectivity of the sensor, we evaluated the potential interference of some of the major known adulterants/diluents found in seized cocaine samples. We tested the interference of 0.5 mmol L^{-1} of lidocaine, benzocaine, phenacetin, procaine, and caffeine in the presence of 0.25 mmol L^{-1} of aminopyrine.

2.3.4. Seized cocaine composition analysis

To characterize the samples of seized cocaine, first we assessed, qualitatively and quantitatively, the presence of adulterants in the samples obtained from the Criminalistics Institute of São Paulo using gas chromatography coupled to a flame ionization detector (GC–FID) according to the procedure reported in the literature [17] in collaboration with the Brazilian National Institute of Criminalistics. The values obtained will be reported in the Results and discussion section.

3. Results and discussion

3.1. Voltammetric measurements

The electrochemical response of aminopyrine was first evaluated using three different working electrodes (gold, glassy carbon, and platinum) to determine which electrode surface provides the highest analytical signal. Cyclic voltammograms were registered using these electrodes in a 0.1 mol L^{-1} phosphate buffer solution containing 2 mmol L^{-1} of aminopyrine. All electrode materials showed a well-shaped faradaic current signal for the aminopyrine oxidation with two clearly electrochemical processes. For better comparison, first, the electroactive area of the electrodes was estimated using a potassium ferrocyanide solution, and the current values of the aminopyrine were normalized by the electroactive area, resulting in a current density (j) value. Fig. 1 shows the voltammograms obtained for the platinum, gold, and glassy carbon electrodes. As we can see, the electrochemical behaviour of aminopyrine independently of the working electrode material shows two oxidation processes. Due to the lower potential of the first peak observed, the current densities of the aminopyrine oxidation for the different working electrode materials were compared in order to determine the material that results in better detection. At 0.35 V, the current density values were 95, 52, and 62 μA mm^{-2} for the platinum, gold, and glassy carbon electrodes, respectively. Thus, the platinum electrode was chosen for the working electrode material as it provided the highest analytical signal of the bare working electrodes tested. Additionally, Fig. 1C reveals that both oxidation processes exhibit an irreversible nature ($I_{pc} / I_{pa} \neq 1$).

3.2. pH study

The oxidation of aminopyrine on the platinum surface was then evaluated at several pH values. The tested electrolytes were as follows: 0.2 mol L^{-1} sulphuric acid (pH = 0.8), 0.1 mol L^{-1} acetate buffer (pH = 4.5), 0.1 mol L^{-1} phosphate buffer (pH = 7.4), and 0.1 mol L^{-1} NaOH (pH = 13). The voltammograms obtained at each pH value are shown in Fig. 2.

At lower pH than 7.4 (Fig. 2B), a new process is observed around 1 V, and the same electrochemical process is noticed using 0.2 mol L^{-1} sulphuric acid (Fig. 2A) as the electrolyte solution. Zhou et al. [7] observed the same behaviour at a pH lower than 7.4, with a broad oxidation peak between 0.3 and 0.8 V and another electrochemical process at a higher potential using a carbon fibre microelectrode. As we are interested in the electrochemical process at the lower potential, *i.e.* less interference for the development of an analytical method, the electrochemical process at the higher potential (>1.0 V) was not explored extensively in this study.

Additionally, it is interesting to highlight that these processes occurring between 0.3 and 0.8 V (Fig. 2B, C, and D) overlap in very acidic conditions (Fig. 2A), showing a strong pH dependency. This behaviour can be explained due to the fact that the solution pH is lower than the pK_a of aminopyrine (5.0 [18]), shifting the first step of aminopyrine