Original scientific paper

A simple and fast method to detect freebase cocaine in artificial saliva by square wave voltammetry (SWV) using carbon paste electrode

Bruno Ferreira, Leandro Oka Duarte, Érica Naomi Oiye,
Maria Fernanda Muzetti Ribeiro, Juliana Midori Toia Katayama,
Pedro Henrique Barros Oliva and Marcelo Firmino de Oliveira$^{\boxtimes}$

GEEQFor – Grupo de Eletroquímica, Eletroanalítica e Química Forense, Departamento de Química - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, 14040-901 - Ribeirão Preto, São Paulo, Brasil

Corresponding author: $^{\boxtimes}$marcelex@usp.br; Tel.: +55 16 3315 9150

Abstract

The consequences of consuming and commercializing illicit drugs including cocaine, constitute a serious problem for authorities and the whole society. Cocaine is usually identified in the laboratory conditions by chromatographic or spectroscopic methods. Electrochemical techniques have also gained prominence because they are fast and easy to use, have many applications, and provide reproducible and reliable results. Therefore, in the present study, a voltammetric method was developed to detect freebase cocaine using carbon paste electrode and methanol as the main cocaine solvent. The developed method was applied to detect cocaine in the artificial saliva by the square wave voltammetry (SWV). The current values increased linearly with the concentration of cocaine, which afforded construction of the analytical curve. The limit of detection (LoD) and the limit of quantifycation (LoQ) were determined as 0.90 µg/mL and 2.41 µg/mL, respectively. For comparison purposes, HPLC-DAD chromatographic method was also applied to detect cocaine. The corresponding analytical curve gave LoD = 0.043 µg/mL and LoQ = 0.130 µg/mL. Although showing better analytical results, HPLC-DAD method could not detect cocaine in saliva samples without previous treatment, what makes the electrochemical method much more attractive for this type of detection.

Keywords
Forensic chemistry; cocaine; saliva; square wave voltammetry

Introduction

Cocaine is a natural substance that is commonly found in *Erythroxylum coca* leaves. This plant is widely cultivated, sold, and distributed in South American countries [1]. Cocaine stimulates the central nervous system and can cause cardiovascular damage, euphoria, and aggressiveness, which has turned it into a worldwide social problem [1,2]. Cocaine can be found in two main forms (Figure 1), cocaine hydrochloride (HCl-salt form) and alkaline cocaine (freebase cocaine) [3].
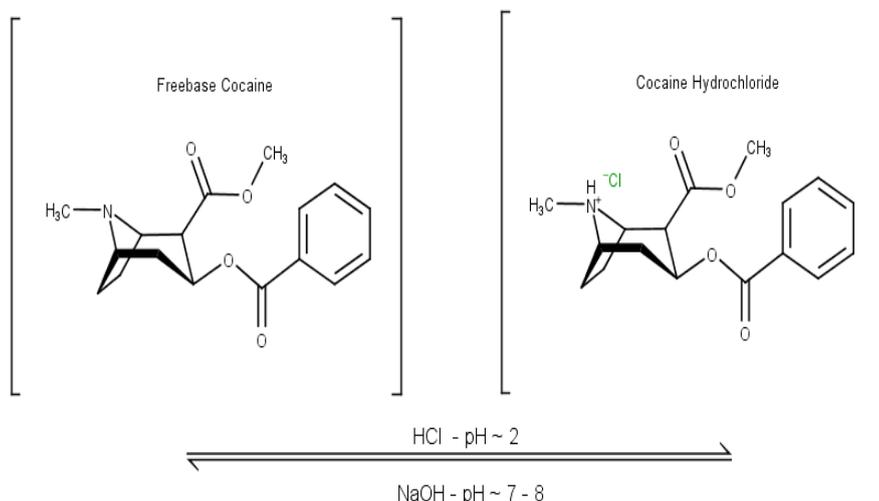
***Figure 1.** Molecular structure difference between freebase cocaine and cocaine hydrochloride.*

The routes of administration of cocaine rely on its chemical form. Freebase cocaine for instance, is usually consumed through smoking, while its salt form is consumed by intranasal or injecting administration [1]. In the human body, the cocaine metabolism can produce some biotransformation products such as benzoylecgonine (BEC), ecgonine (ECG), and ecgonine methyl ester (EME) [4]. The cocaine has half-life of approximately one hour [1-3]. When consumed, this drug can concentrate in the tissues, increasing its detection window. Currently, there are various literature studies involving cocaine detection in biological samples such as urine, plasma, and saliva [3].

In the saliva, cocaine may be available up to 48 h after administration, and its concentration in this fluid is higher than the concentration of its main metabolite, benzoylecgonine [5,6]. The use of saliva to detect narcotics is advantageous over other biological fluids, because saliva is easy to be sampling, has lower concentration of proteins and endogenous compounds than urine or blood, and its adulteration and contamination are less likely to occur since collection is usually done under supervision [7]. Therefore, government agencies use this matrix in the case of traffic accidents, for toxicological monitoring of employees and even for high-performance sports (*e.g.* anti-doping tests) [6]. On the other hand, the use of saliva has limitations like small volume for detection, high possibility of contamination by exogenous substances (*e.g.* nicotine and food residues) and high viscosity [6,7].

Chromatographic techniques, especially gas or liquid chromatography coupled with mass spectrometry (MS), are commonly used to detect cocaine in saliva and other fluids because of their high sensitivity and selectivity [8]. Indeed, these techniques are often employed because of the biological matrix complexity and lower concentrations of the analytes. Preparing the sample for injection into the chromatographic system is another important point. Since these processes are generally long and costly, the search for new reliable methods that can detect cocaine and other narcotics in biological samples becomes an urgent matter [5,8].

Beside the chromatographic techniques, cocaine and its metabolites have already been accomplished using voltammetric techniques [4,9,10]. The direct electrochemical determination of cocaine is widely performed in seized samples using a wide range of electrodes and voltammetric techniques (*e.g.* square wave voltammetry and cyclic voltammetry). For detection of its metabolites, few works have already appeared in the literature, where the main ones used mercury drop electrode as the working electrode [4,9,10]. On the other hand, there is an apparent lack of research involving determination of cocaine in biological samples. In this specific area of toxicological analyses, the voltammetric techniques have high potential, since they have good reproducibility, high sensitivity and precision, and no need for biological sample pre-treatment procedures [11-14].

It has already been shown that when carbon paste electrode was used as the working electrode for cocaine detection, the anodic scan refers to the oxidation of tertiary amine in cocaine molecule [11,15]. An intermediate, imine, originates from this oxidation on the electrode surface when two electrons and one proton are lost [11,15]. Freebase cocaine is oxidized at potentials close to 1.1 V *vs*. Ag/AgCl [15]. In the forensic field, cocaine detection is usually accomplished in countless seized samples, but few studies have been dedicated to the direct procedure of cocaine detection in biological matrices, particularly saliva [11].

This work aims to detect freebase cocaine in artificial saliva by a direct method that uses a SWV technique and unmodified carbon paste electrode. This procedure is advantageous because it allows simple and rapid cocaine detection based on the cocaine amine group oxidation [11]. This work also compares the electrochemical results obtained by SW voltammetry with the results obtained by the high-performance liquid chromatography (HPLC-DAD) as one of the most frequently applied techniques for this purpose.

Experimental

Materials and reagents

Methanol and acetonitrile, both HPLC grade, were purchased from Honeywell. The other reagents were analytical grade. Nitric acid, sodium phosphate dibasic (Cinética Química), potassium carbonate (Sigma Aldrich) and the salts of sodium (chloride, nitrite and hydroxide), obtained from Merck, were used to prepare artificial saliva. For preparing supporting electrolyte (pH 9.5), the salts of perchlorate (Synth) and potassium phosphate dibasic (Sigma Aldrich) were used. Mixture of graphite powder (Fisher Scientific) and paraffin (Isogama) were used to prepare the carbon paste electrode. For analyses of possible interference, stock solutions of caffeine at 485 µg/mL and lidocaine at 586 µg/mL were prepared by dissolving the respective standard powder in methanol. Cocaine powder (99.8 % w/w) was acquired from the laboratory of toxicological analysis – Institute of Criminalistics – Ribeirão Preto, State of São Paulo, Brazil and purified following a procedure described in the literature [12]. After this step, the stock solution of cocaine at 760 µg/mL was prepared by dissolving cocaine purified powder in methanol.

Apparatus

Electrochemical measurements were performed by the square wave voltammetry (SWV) technique. µ-Autolab III potentiostat coupled to a microcomputer with NOVA software v. 1.11.2 (Metrohm Autolab) was employed.

Gas chromatograph coupled to a mass spectrometer (GC/MS) was conducted on a Shimadzu model QP2010SE (Nakagyo-ku, Kyoto, JPN) apparatus coupled to a single quadrupole mass

spectrometer with electron impact ionizer. The analysis was carried out in an RTX-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from Restek Corporation (Bellefonte, PA, USA).

An automated liquid chromatography coupled with diode array detector (LC/DAD) system by Thermo Scientific Dionex model Ultimate 3000 HPLC SQ (Waltham, MA, USA) equipped with an LPG-3400SD quaternary pump, a WPS-3000RS autosampler, a TCC-3000SD column compartment, a DAD-3000RS UV diode detector, and a C8 column (250 × 4.6 mm, 5 μm particle size) (NanoScience Technologies) were used.

Manufacture of carbon paste electrode

Carbon paste electrode (CPE) was prepared using 3 g of graphite powder with 3 g of paraffine (50:50 w/w). Graphite and paraffine were homogenized at high temperature (about 100 °C) until the paste was formed. Afterwards, carbon paste was packed into a plastic cylindrical tube of a syringe form (Figure 2). At the end of each measurement, the electrode surface was cleaned by a polishing paper.

Figure 2. *Carbon paste electrode prepared and employed in this research*

Preparation and fortification of artificial saliva

Artificial saliva was prepared as follows: sodium hydrogen carbonate (4.20 g), sodium chloride (0.50 g), potassium carbonate (0.20 g), and sodium nitrite (0.03 g) were weight and put into a volumetric flask of 1000 mL with purified water and pH was adjusted to 5 with nitric acid [16]. Next, the artificial saliva was fortified with the standard of cocaine (760 μg/mL) previously purified as described above. The final concentration of cocaine in the electrochemical cell was 10 μg/mL.

GC/MS analysis

GC/MS was used to evaluate the presence of other adulterant molecules commonly found in the seized cocaine. This step is considered important because the seized cocaine was purified and the presence of adulterants or interferents in the sample could interfere with the electrochemical signal of cocaine. A solution of 20 μg/mL cocaine was prepared by diluting the stock solution with methanol, and 1 μL of this solution was injected in the splitless mode. The analysis conditions were 150 °C for 1 min, heating at 20 °C min^{-1} up to 280 °C, isotherm for 4 min, and heating at 10 °C min^{-1} up to 300 °C for 2 min. The injector and interface temperatures were 250 and 300 °C, respectively. The solvent delay was 2.5 min, and the ionization source was electron ionization (IE) operated at 70 eV. The spectra were obtained in the full scan mode.

Squared wave voltammetry analysis

Square wave voltammetry (SWV) measurements were accomplished with a conventional three-electrode cell of 5 mL capacity. Carbon paste electrode (50:50 w/w) served as the working electrode,

Ag/AgCl electrode with aqueous saturated KCl as the reference electrode, and a platinum spiral served as the auxiliary electrode. SWV method was optimized in the supporting electrolyte solution of 0.1 mol/L NH_4ClO_4 and 0.1 mol/L K_2HPO_4 by varying pH adjusted by sodium hydroxide solution to 8.0, 9.5 and 10.5, pre-concentration time (0, 30, 60, 90 and 120 s), pre-concentration potential (0.8, 0.9, 1.0, 1.1, and 1.2 V), frequency (25, 50, 75, 100, 150 and 150 Hz), and amplitude (0.01 to 0.50 V). After the optimization step, the analytical curve was constructed by employing five concentration levels of cocaine: 2.54, 5.08, 7.62, 10.11, and 15.16 µg/mL.

The voltammetric response using SWV of freebase cocaine in artificial saliva was performed with the fortification of 10 µg/mL cocaine in the saliva solution and using optimized values of experimental parameters determined for freebase cocaine.

HPLC-DAD analysis

HPLC-DAD method was developed after the following parameters were evaluated: mobile phase composition (water/acetonitrile or methanol), pH (3.5 and 5.0), flow rate (0.6 and 1.0 mL min^{-1}), detection wavelength (195, 235, and 275 nm), and temperature (24, 35, and 45 °C). The pH was adjusted with 0.1 % (v/v) phosphoric acid and triethylamine. The injection volume was fixed at 25 µL. The analytical curve was plotted by using six concentration levels of cocaine: 1.0, 2.0, 5.0, 10.0, 20.0, and 50 µg /mL.

Limits of detection (LoD) and quantification (LoQ)

The analytical parameters for both applied techniques were calculated from the corresponding analytical curves generated by the optimized methods. The analytical parameters are: method sensitivity, linearity range, and limits of detection (LoD) and quantification (LoQ). LoD and LoQ are defined as: LoD = 3SD/m and LoQ = 10SD/m, where m is the sensitivity (slope) of the analytical curve and SD is the standard deviation of the response.

Results and discussion

Cocaine purity evaluation by GC/MS analysis

From the gas chromatogram correlating the chromatographic peak with the mass spectrum (GC/MS) of freebase cocaine in methanol shown in Figure 3, it can be concluded that there are no other organic compounds present in the sample. This would be important, because presence of other organic compounds may interfere with the cocaine electrochemical signal, *i.e.* these molecules can have anodic oxidation peak at the same potential as cocaine oxidation.

Cocaine detection method optimization by SWV analysis

After verifying the absence of any organic compounds in the previously purified cocaine using GC-MS, an initial screening analysis of freebase cocaine (10 µg/mL) was determined by SWV. The obtained voltammetric signal was then compared to the voltammetric signals of the supporting electrolyte (0.1 mol/L NH_4ClO_4 and 0.1 mol/L K_2HPO_4, pH 9.5), and pure methanol (Figure 4). In these measurements, SWV experimental parameters (amplitude signal, frequency, pre-concentration time, and pre-concentration potential) were not optimized. The cocaine voltammogram in Figure 4 shows that the electrochemical response emerged at 1.08 V. This potential value is different from that of cocaine hydrochloride, because this form of salt has been protonated with addition of HCl and the potential for its oxidation must be higher compared to the other molecular form [17]. In other words, because the cocaine in the salt form has already been protonated, more energy is needed for its oxidation compared to the freebase cocaine form.
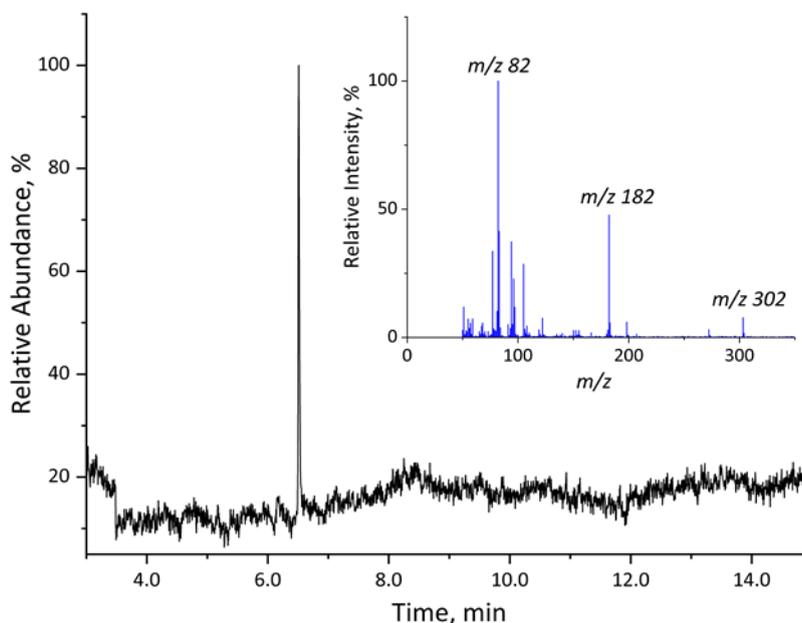
Figure 3. *Gas chromatogram of powder freebase cocaine in methanol and its respective mass spectrum showing cocaine as the only detectable molecule.*
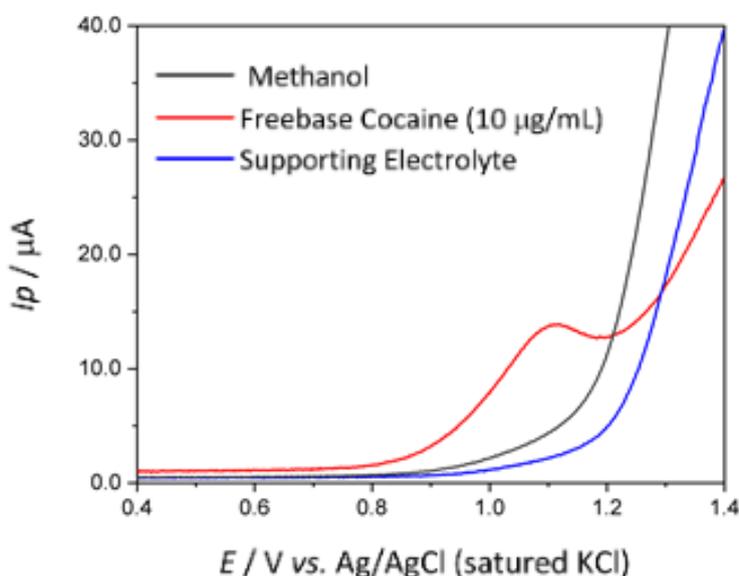
Figure 4. *Screening of freebase cocaine (10 µg/mL) detection on carbon paste electrode using SWV in 0.1 mol/L NH₄ClO₄ and 0.1 mol/L K₂HPO₄ as supporting electrolyte (red). SW voltammograms of 40 µL pure methanol (black) and pure supporting electrolyte (blue). Experimental SWV parameters: f =100 Hz, A = 0.010 V, pre-concentration time of 60 s at 1.0 V.*

After the initial screening analysis of freebase cocaine, the supporting electrolyte solution pH (pH$_{SE}$) and SWV experimental parameters were optimized by varying pH, amplitude signal, frequency, pre-concentration time, and pre-concentration potential. Initially, pH variation of the supporting electrolyte showed the highest oxidation peak at pH 9.5. Therefore, this basic pH value for the supporting electrolyte was chosen for further experiments, and also because in acid pH, cocaine molecule could be protonated and more energy would be needed for oxidation of that form of cocaine. After pH optimization, the experimental SWV parameters cited above where varied and optimized on the basis of *the lowest potential and the highest current* criteria. Table 1 lists the optimized values of SWV experimental parameters that were applied for construction of the analytical (calibration) curve.

Table 1. *Optimized values of SWV experimental parameters for construction of freebase cocaine analytical curve*

Experimental SWV parameter values	
pH_{SE}	9.5
Amplitude, V	0.1
Frequency, Hz	75
Pre-concentration time, s	30
Pre-concentration potential, V	1.0

SWV analysis

SW voltammograms and the analytical curve for cocaine, obtained by SWV technique with the optimized experimental parameters (Table 1) are shown in Figure 5. Figure 5a shows the measured SWVs and reveals that the current increased with increasing freebase cocaine concentration. Figure 5b illustrates the analytical curve with the corresponding deviations set for the measured current at each concentration. LoD and LoQ values were determined as 0.80 and 2.41 µg mL^{-1}, respectively, while the correlation coefficient was equal to 0.9992. The analytical parameters determined from the analytical curve in Figure 5b are summarized in Table 2.
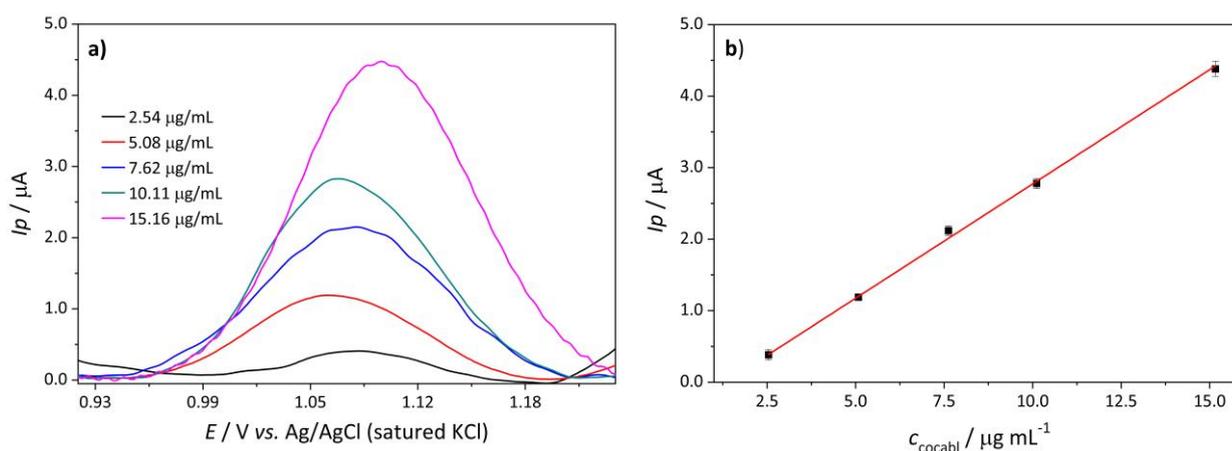
Figure 5. *(a) SW voltammograms of freebase cocaine (concentrations: 2.54, 5.08, 7.62, 10.11, and 15.16 µg/mL) in the supporting electrolyte (0.1 mol/L NH$_4$ClO$_4$ and 0.1 mol/L K$_2$HPO$_4$, pH 9.5) and (b) relation between peak current and concentration of freebase cocaine. SWV experimental parameters are given in Table 1*

Table 2. *Values of analytical parameters obtained from the analytical curve constructed for freebase cocaine using SWV technique*

Parameter	Values
LoD, µg/mL	0.90
LoQ, µg/mL	2.41
Linear range, µg/mL	2.54 – 15.2
Linearity[a]	0.9992
Sensitivity, A mL µg^{-1}	3.15×10^{-7}

[a] Pearson correlation coefficient

Study of interfering substances

The study of interfering substances was conducted with caffeine (CAF, 10 µg/mL) and lidocaine (LID, 10 µg/mL) present in the supporting electrolyte, using the SW voltammetry method developed in this work. The SW voltammogram in Figure 6a refers to caffeine (black line) and shows that no oxidation peak emerged at 1.08 V. When freebase cocaine (COC, red line) was added to the solution already containing caffeine, the oxidation peak at about 1.10 V appeared, suggesting that caffeine

does not interfere with the voltammetric response of cocaine. This is supported by literature data showing also that in SWV measurement of cocaine on unmodified carbon paste electrode, caffeine is not an interfering compound [19-21]. SW voltammogram presented in Figure 6b (black line) refers to lidocaine (10 µg/mL). Two oxidation peaks are displayed at 1.20 V and around 0.9 V, respectively. When freebase cocaine (10 µg/mL) was added to the solution already containing lidocaine, SW voltammogram (red line) showed that the oxidation peak at 0.9 V was fully suppressed, leaving the peak at 1.14 V to be the single oxidation peak.
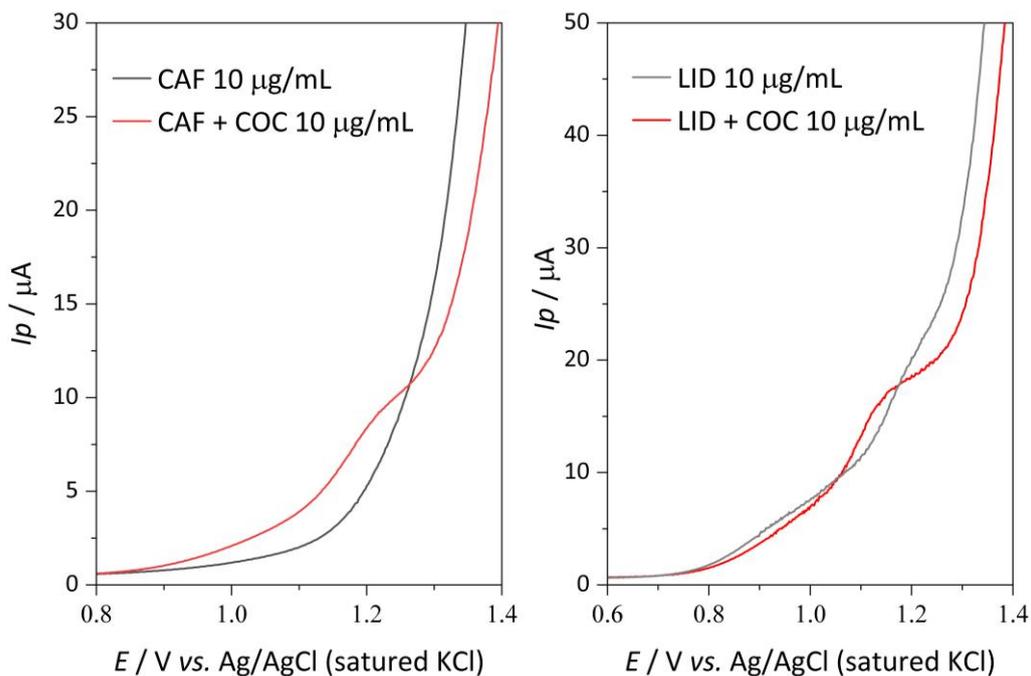
Figure 6. *SW voltammograms of two main cocaine adulterants: (a) black voltammogram represents 10 µg/mL caffeine, and red voltammogram represents 10 µg/mL caffeine with addition of 10 µg/mL freebase cocaine (1:1). (b) black voltammogram represents 10 µg/mL lidocaine, and red voltammogram represents 10 µg/mL lidocaine with addition of 10 µg/mL freebase cocaine (1:1). Supporting electrolyte: 0.1 mol/L NH_4ClO_4 with 0.1 mol/L K_2HPO_4, pH 9.5. SWV experimental parameters are given in Table 1*

HPLC-DAD method and analytical curve construction

To compare the results of cocaine determination obtained by SW voltammetric method, the chromatography method (HPLC-DAD) was applied additionally. The optimized experimental parameters of HPLC-DAD method for the freebase cocaine detection are summarized in Table 3.

Table 3. *Optimized experimental parameters used to construct HPLC-DAD analytical curve*

Parameter	Results
Column	C8
Mobile phase	Water: Acetonitrile (75:25)
Additions	0.1% phosphoric acid and Triethylamine (pH 3.5)
Flow, mL min^{-1}	1.0
Column temperature, °C	25
Wavelength, nm	195
Injection volume, µL	25

The constructed analytical curve is shown in Figure 7, while the corresponding analytical parameters for freebase cocaine detection are summarized in Table 4.
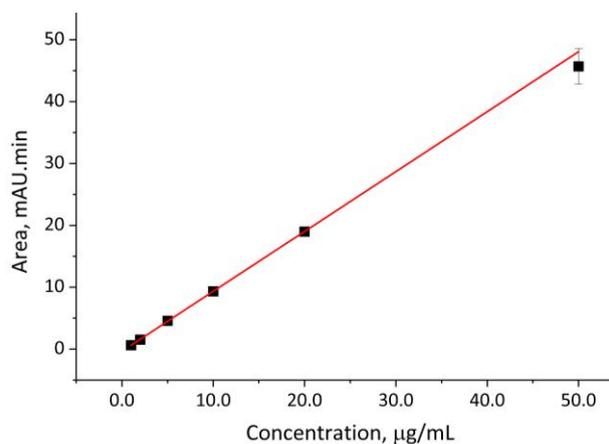
Figure 7. *HPLC-DAD analytical curve for freebase cocaine (concentrations: 1.0, 2.0, 5.0, 10.0, 20.0, and 50.0 μg/mL. Optimized experimental parameters are given in Table 3*

Table 4. *Values of analytical parameters obtained from the analytical curve constructed for freebase cocaine using HPLC-DAD technique*

Parameter	Values
LoD, μg/mL	0.043
LoQ, μg/mL	0.130
Linear range, μg/mL	1.0 - 50
Linearity[a], r	0.9996
Sensitivity, mAU mL μg^{-1}	0.97

[a] Pearson correlation coefficient

Identification of freebase cocaine fortified in artificial saliva using SWV

Once the SW voltammetric method for cocaine detection was developed, the analysis of artificial saliva fortified with freebase cocaine is performed. Freebase cocaine was detected in the artificial saliva by using SWV technique with optimized voltammetric parameters given in Table 1. Figure 8 illustrates SW voltammograms for the freebase cocaine present in the artificial saliva and the analytical curve for freebase cocaine (Figure 5b). Figure 8a represents SW voltammogram of the supporting electrolyte (0.1 mol/L NH_4ClO_4 with 0.1 mol/L K_2HPO_4, pH 9.5) containing artificial saliva only. Figure 8b reveals the detection of 10 μg/mL freebase cocaine fortified artificial saliva in the supporting electrolyte. It is seen that an anodic peak emerged at the same potential (about 1.10 V) observed for a purified freebase cocaine standard (Figure 4). Figure 8c shows the analytical curve from which the concentration of freebase cocaine can be estimated from the current obtained for the saliva spiked with 10 μg/mL freebase cocaine.

The peak current value measured in the solution containing artificial saliva and fortified with freebase cocaine (1.77 μA) was lower than the current value achieved with the freebase cocaine standard. This may happen due to the high concentration of various ions of different sizes in the artificial saliva solution, which may compete with cocaine for interaction with the active sites present on the carbon paste electrode surface [22]. Also, the optimization of the method performed for cocaine fortified in artificial saliva would possibly result by increase of the anode current. Despite the decreased current, however, the voltammetric response is relatively quick because there was no need for some type of pre-treatment to make the analyte available for SWV analysis. Contrary to that, when detection of cocaine fortified in artificial saliva was carried out using the chromatographic method, the salt contained in the artificial saliva should be eliminated due to experimental limitation of this technique, what resulted in more costs and longer analysis time.
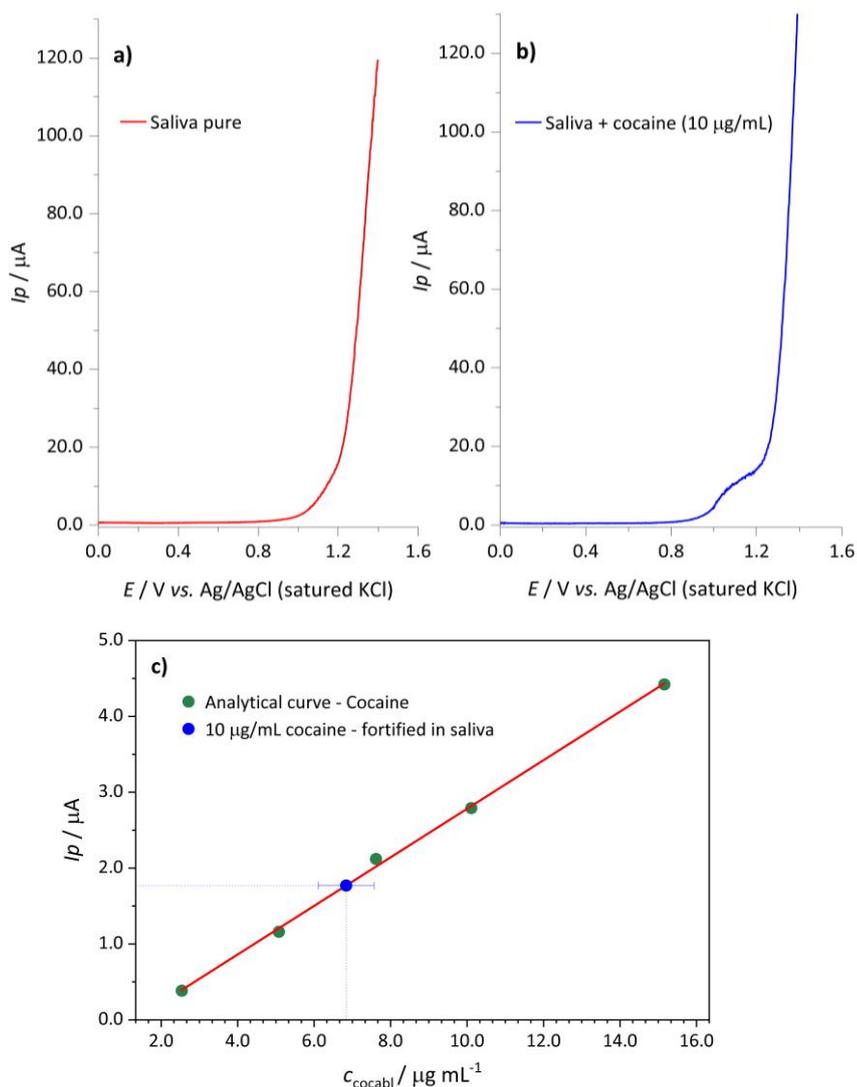
Figure 8. *SW voltammograms of (a) pure artificial saliva and (b) artificial saliva containing 10 μg/mL of freebase cocaine. (c) Anodic peak current of 10 μg/mL freebase cocaine (1.77 μA) is denoted as blue point in the analytical curve of freebase cocaine. Supporting electrolyte: 0.1 mol/L NH_4ClO_4 and 0.1 mol/L K_2HPO_4, pH 9.5. SWV experimental parameters are given in Table 1*

Conclusions

The fast and efficient procedure is developed for the squared wave voltammetric (SWV) detection of freebase cocaine in artificial saliva, using the manufactured carbon paste electrode. The carbon paste electrode manufacture is easy and does not require expensive materials, what reduces the overall analysis cost. The electrochemical response of cocaine in the artificial saliva is in agreement with the literature, *i.e.*, the oxidation potential of the tertiary aliphatic amine in the cocaine molecule is found at about 1.08 V *vs*. Ag/AgCl electrode. The selectivity for lidocaine and caffeine that are often found in cocaine samples, has been evaluated and confirmed. This opens up the possibility for future analysis of cocaine in real samples of saliva and other biological matrices.

Even though the values of LoD and LoQ obtained by SWV are worse than those obtained with HPLC-DAD technique, here established method shows certain advantages, such as simplicity, speed, less use of solvent, low-cost materials, and no need for sample preparation (liquid-liquid (LLE) and solid phase extraction (SPE)). All these make the electrochemical technique accessible to many laboratories where its application could reduce waste generation and costs.

References

[1]   R. A. Goldstein, C. DesLauriers, A. Burda, H. Johnson-Arbor, *Seminars in Diagnostic Pathology* **26(1)** (2009) 10–17.

[2]   C. M. Shanti, C. E. Lucas, *Critical Care Medicine* **31(6)** (2003) 1851–1859.

[3]   N. G. Oliveira, R. J Dinis-Oliveira, *Archives of Toxicology* **92(10)** (2018) 2987–3006.

[4]   V. Pavlova, V. Mirčeski, Š. Komorsky-Lovrić, S. Petrovska-Jovanović, B. Mitrevski, *Analytica Chimica Acta* **512(1)** (2004) 49–56.

[5]   R. A. Jufer, A. Wstadik, S. L. Walsh, B. S. Levine, E. J. Cone, *Journal of Analytical Toxicology* **24** (2000) 467–477.

[6]   W. M. Bosker, R. A. Huestis, *Clinical Chemistry* **55(11)** (2009) 1910–1931.

[7]   K. R. Allen, *Annals of Clinical Biochemistry* **48** (2011) 531–541.

[8]   D. V. M. Souza, F. V. Pereira, C. C. Nascentes, J. S. Moreira, V. H. M. Boratto, R. M. Orlando, *Talanta* **208** (2020). 120353. https://doi.org/10.1016/j.talanta.2019.120353

[9]   Š. Komorsky-Lovrić, S. Gagić, R. Penovski, *Analytica Chimica Acta* **389(1-3)** (1999) 219–223.

[10]   Š. Komorsky-Lovrić, I. Galić, R. Penovski, *Electroanalysis* **11(2)** (1999) 120–123.

[11]   L. Poltorack, E. J. R. Sudhölter, M. de Puit, *Trends in Analytical Chemistry* **114** (2019) 48–55.

[12]   E. N. Oiye, N. B. de Figueiredo, J. F. de Andrade, H. M. Tristão, M. F de Oliveira, *Forensic Science International* **192(1-3)** (2009) 94–97.

[13]   V. Vukojević, S. Djurdjic, Ľ. Švorc, T. Ćirković Veličković, J. J. Mutić, D.M. Stanković, *Food Analytical Methods* **11(9)** (2018) 2590–2596.

[14]   O. Sarakhman, S. Pysarevska, L. Dubenska, D. M. Stanković, P. Otřísal, A. Planková, K. Kianičková, Ľ. Švorc, *Journal of the Eletrochemical Society* **166(4)** (2019) B219–B226.

[15]   M. T. F. Abedul, J. R. B. Rodriguez, A. C. García, P. T. Blanco, *Electronalysis* **3(4-5)** (1991) 409–412.

[16]   M. Dronova, E. Smolianitski, O. Lev, *Analytical Chemistry* **88(8)** (2016) 4487– 4494.

[17]   J. M. Freitas, D. L. O. Ramos, R. M. F. Souza, T. R. L. C. Paixão, M. H. P. Santana, R. A. A. Muñoz, E.M. Richter, *Sensors and Actuators B* **243** (2017) 557–565.

[18]   A. Warner, A. B. Norman, *Therapeutic Drug Monitoring* **22(3)** (2000) 266–270.

[19]   B. J. Sanghavi, A. K. Srivastava, *Eletrochimica Acta* **55** (2010) 8638–8648.

[20]   E. Molaakbari, A. Mostafavi, H. Beitollahi, *Sensors and Actuators B: Chemical* **208** (2015) 195–203.

[21]   G. A. M. Mersal, *Food Analytical Methods* **5** (2012) 520–529.

[22]   R. L. McCreery, *Chemical Reviews* **108** (2008) 2646–2687.