A simple method to detect the stimulant modafinil in authentic saliva using a carbon-nanotube screen-printed electrode with adsorptive stripping voltammetry.

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Abstract: Electrochemical detection of modafinil, a stimulant banned in sports competitions, is reported for the first time. An electrochemical sensor is developed based on carbon nanotubes screen-printed electrodes (SPE-CNT) with adsorptive stripping wave voltammetry (AdSWV). The proposed electrochemical method is applied to authentic human saliva for modafinil determination (LOD of 2.0 µM) without interference from uric acid or ascorbic acid. The analytical performance of the SPE-CNT with AdSWV for detection of modafinil in authentic saliva samples suggests its possible application as a simple and fast method for doping control.

Keywords: *Adsorptive stripping voltammetry; Carbon nanotubes; Doping control; Modafinil Saliva; Screen-printed electrode.*

1. Introduction

1.1 Screen Printed Electrode and Adsorptive Stripping Voltammetry

Screen-printed electrodes (SPEs) have been widely used in electroanalysis due to their simplicity of application in many analytical chemistry tasks; for recent reviews see references [1-10]. These reviews survey some of the more important papers from over 1500 publications, which the Web of Science™ database generates using the key words "screen-printed electrode" in the title search. This massive number of papers using SPEs is partly explained by the fact that these sensors are disposable devices. This advantage avoids the tedious polishing (or electrochemical treatment) step to reuse the working electrode that is required for most solid electrodes to overcome passivation or/and contamination on their surfaces. In addition, SPEs provide portability to electrochemical detection enabling the use of only a small volume of sample in the microliter range.

The manufacturing of SPEs is based on printing technology and microelectronic devices, which offer a large diversity of SPEs with adequate reproducibility for electrochemical detection and a low cost depending on the material [6, 9]. SPE working electrodes based on carbon are the most widely used and are based on mainly graphite (SPE-Gr), graphene or multi-wall (or single-wall) carbon-nanotubes (SPE-CNT) [1-11]. Carbon SPEs are often chosen due to their high sensitivity for electrochemical detection, especially, when the target adsorbs on the electrode surface. Although SPE-Gr offers a lower cost than SPE-CNT, carbon-nanotubes as working electrodes generally provide more sensitivity in electroanalysis due to their higher surface area [6] for techniques including adsorption of analytes.

Adsorptive stripping voltammetry (AdSV) is one of the more sensitive electroanalytical techniques, and consists of two steps. First a pre-accumulation step of the analyte on the working electrode surface by an adsorption process (physical or chemical) followed by an electrochemical detection step, which can be performed using different voltammetry modes for oxidation or

reduction of the accumulated substance. The use of the AdSV technique using several different electrodes has summarized in a recent paper [12]. AdSV using SPEs benefits from the simplicity of application as reported in many electroanalytical methods [13-20] for example, allowing a good performance for determination of substances in biological samples as urine [15-17] and serum [13, 18, 19], but never hitherto in saliva. Furthermore, most of these methods have been applied in diluted (or synthetic) biological samples. In contrast, this work presents the use of SPE-CNT with the AdSV technique to detect the stimulant modafinil in *undiluted authentic saliva*. Note that conclusions drawn from synthetic ("fake") saliva studies are often doubtful [21]. Modafinil is a stimulant banned in the doping control and of interest to the World Anti-Doping Agency (WADA) [22].

1.2 Detection of Modafinil and Doping Control in Saliva

Modafinil (Fig. 1) is used as a stimulant for treatment of excessive daytime sleepiness, which is related to several sleep disturbances such as restless leg syndrome and narcolepsy [23]. Furthermore, although the modafinil has shown to be addictive, this drug has been suggested for the treatment of cocaine addiction [23]. Due to its stimulant effects some doping cases have been reported from modafinil intake before or during competitions [24]. The most famous of them was in the 2003 world track and field championship when an American sprinter was disqualified in the 100 m victory due to a positive test for modafinil [24]. In this context, modafinil determination has been reported in biological fluids and pharmaceutical formulations by several methods as surveyed in a recent review [25], where the most of them are based on high performance liquid chromatography (HLPC) with UV or mass spectrometry detection.

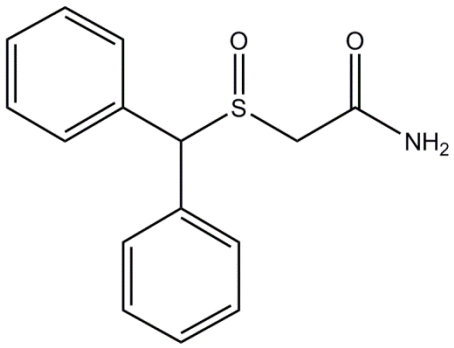


Fig. 1. Molecular structure of modafinil

Anti-doping analysis has been performed in urine samples and is regulated by WADA, which publishes an annual list of prohibited drugs [22]. In addition, the concomitant use of saliva and urine samples offers an improvement to anti-doping analysis, allowing the correlation between the unchanged drug in saliva with its metabolites in urine, as reported for the stimulant modafinil and for others [26]. This correlation is important as an auxiliary in a positive test for banned drugs by WADA as well as to clarify whether a drug had been taken by the athlete for a short or a long time before competition. Due to the low levels of the non-metabolised forms in urine or blood, the detection of these analytes [26] in saliva is easier than in other samples.

Furthermore, due to the simple and non-invasive collection, the saliva sample is a more viable approach to application of screening methods for doping control. Several analytical methods have been widely used for drug detection and disease diagnosis using saliva samples [21], but most notably chromatographic methods with mass spectrometry detection. Nevertheless, electroanalytical methods offer easy *on-site* analysis or screening of saliva samples [21]. However, too few methods have explored the application in undiluted human saliva [12, 27, 28]. Portable and disposable electrochemical sensors such as SPEs provide a simple and attractive prospective method to anti-doping analysis in authentic saliva.

In this context, we present a simple electrochemical procedure for modafinil determination in authentic human saliva using a SPE-CNT with AdSV. It is worth mentioning that this work presents, for the first time, the use of SPE-AdSV for substance detection in authentic saliva samples. The proposed method provides a good analytical performance for possible application in doping control using saliva samples containing prohibited levels of modafinil.

2. Experimental

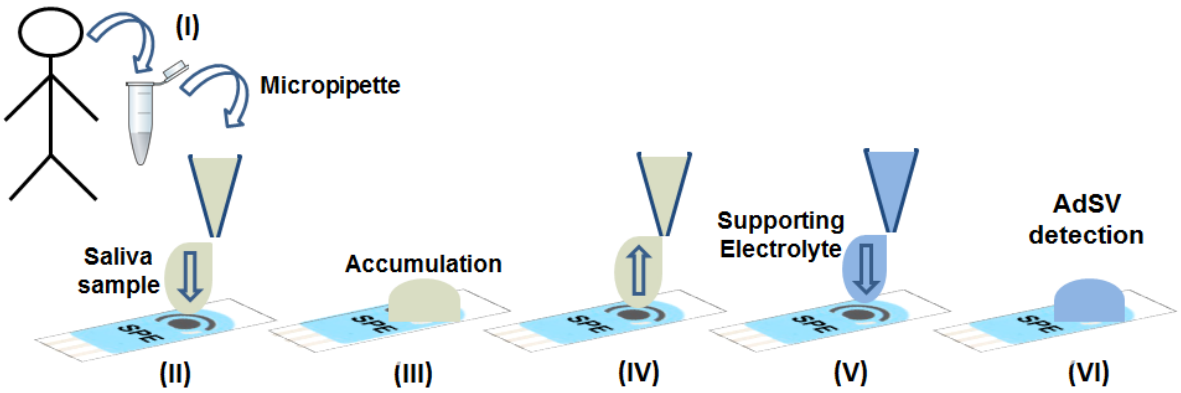
2.1. Instrumental and apparatus

A µAutolab III potentiostat / galvanostat (Eco Chemie, the Netherlands) controlled by NOVA software 2.0, was used to perform the voltammetric experiments. All electrochemical measurements were carried out in a Faraday cage thermostatted at 25 ^{o}C. A pure nitrogen gas was bubbled to remove oxygen from all the studied solutions using an electrochemical cell of three electrodes. A glassy carbon electrode (GCE, BAS Technical, USA), with a geometric area of 0.068 cm^2, was used as the working electrode for initial studies of the electrochemical behavior of the analyte. The reference and counter electrodes used a saturated calomel electrode, SCE (SCE +0.244 V vs. SHE, BASiInc., Japan) and a graphite rod, respectively. Voltammetric measurements were performed in conventional glass cell (1.0 mL). The scan rate (10 to 800 mV s^{-1}) study was conducted on 1.0 mM modafinil in 0.1 M H_2SO_4 at the GCE. A carbon microdisc (36 µm diameter, BASi) was used to investigate the possible mass-transport control of the modafinil oxidation in electrochemical detection.

Commercial carbon SPEs from Dropsens (Oviedo, Spain) were used for modafinil determination studies. According to the company literature [29] the working electrodes on SPEs are based on carbon graphite (SPE-Gr, model DRP-110) and carbon nanotubes (SPE-CNT, model DRP-110CNT) and both have a 4-mm diameter carbon disc. In these SPE devices, carbon and silver

are also printed on the same SPE base and are used as auxiliary and pseudo-reference electrodes, respectively. The SPEs were stored at room temperature with no special precautions and used as purchased. The electrochemical measurements on SPE were performed by drop-casting of 100 µL of an electrolyte or sample solution to cover all electrodes. Prior to each measurement, all SPE were electrochemically conditioned in 0.1 M H_2SO_4 by cyclic voltammetry (10 scans) at a scan rate of 100 mV s^{-1} from -1.2 to +1.6V. Voltammetry was carried out in the drop-casted solution on the SPE. Cyclic voltammograms at a scan rate of 50 mV s^{-1} were obtained for electrochemical studies of 1.0 mM modafinil in different pH values (0.3 to 12) using H_2SO_4 and phosphate solutions as supporting electrolytes for the GCE and the SPE-CNT.

AdSV was investigated for modafinil detection in 0.1 M H_2SO_4 and directly in the authentic saliva samples. The accumulation time (2.0 to 30 min) for modafinil detection in 0.1 M H_2SO_4 was optimized. As illustrated in Scheme 1, the analysis in authentic saliva was carried out according to following steps: (I) saliva samples were collected using Salivetts®, see section 2.3; (II) the saliva samples (100 µL) with or without addition of modafinil were drop-casted on SPE-CNT using a micropipette; (III) the accumulation step required for AdSV was carried out directly in authentic saliva samples (undiluted) from which the analyte was extracted by adsorption on the electrode; (IV) the saliva samples were removed using a micropipette; (V) the supporting electrolyte was added and; (VI) the detection was performed by AdSV or adsorptive square-wave voltammetry (AdSWV) techniques. The frequency, amplitude and step potential were optimized for AdSWV detection. The determination of 5.0 to 500 µM modafinil by AdSWV was performed with the pre-accumulation step in 0.1 M H_2SO_4 and in authentic saliva at SPE-CNT. The reproducibility study was performed for modafinil determination in authentic saliva using different SPEs-CNT. The obtained curves by AdSWV detection were baseline corrected using a polynomial (order 4) to fit the rising background current before subtraction. This same procedure was used to calculate all the peak areas obtained from the modafinil oxidation process.



Scheme 1. Analysis in authentic saliva samples by the AdSV technique using a SPE-CNT. Saliva samples were collected (I) using Salivetts®, see section 2.3.

2.2. Chemicals and solutions

Ultra-pure deionized water (resistivity not less than 18.2 MΩ cm at 25 ^{o}C) from a Milli-Q system (Millipore, USA) was used to prepare all the solutions. Modafinil (2-[(diphenylmethyl)sulfinyl]acetamide) was purchased from Tocris Bioscience (Bristol, UK). The stock solution of 1.0 x 10^{-2} M modafinil was prepared in ethanol (100%) from Sigma-Aldrich (Lancashire, UK). The same percentage of ethanol was added to the supporting electrolyte for all voltammetric studies of the modafinil. Sulfuric acid, sodium hydroxide, phosphoric acid monobasic and dibasic sodium phosphates were all of analytical grade and were purchased from Sigma-Aldrich (Lancashire, UK). Phosphate buffer solution (PBS) was prepared at a 0.1 M concentration in different pHs (2 to 12). Sulfuric acid solutions were prepared for electrochemical behaviour studies of the modafinil in stronger acid medium at the concentration of 0.02 to 0.50 M with the measured pH between 0.3 to 1.5.

2.3. Saliva sample preparation

Respecting all guidelines for working with authentic human saliva [21], samples were collected from healthy volunteers using Salivetts® (Sarstedt, Germany). Each volunteer chewed the swabs from the Salivetts® by for 1 min and then two Salivetts® containing the respective swabs were centrifuged at 2800 rpm for 5 min. Then, 1.0 mL saliva samples were transferred to the conventional electrochemical cell of three electrodes. For SPEs measurements 100 µL of the saliva sample was used by drop-casting on working electrode surface of this device. The measurements in authentic saliva samples were performed before and after addition of modafinil standard solutions. The interference study was performed by addition of common compounds found in the authentic human saliva [21] in which the electrochemical signal of 50 µM modafinil was evaluated before and after addition of these interferents. In particular the interference of uric acid (> 99%, Sigma-Aldrich) and L-ascorbic acid (> 99%, Sigma-Aldrich) was studied.

3. Results and discussion

In the following, first, the electrochemical behavior of modafinil is investigated in section 3.1. Using a GCE, as well as a carbon microelectrode to evaluate some parameters such as the optimal supporting electrolytes, pH and the possible mass-transport control of the electrochemical reaction. The use of SPEs based on graphite (SPE-Gr) and carbon nanotubes (SPE-CNT) is also studied to facilitate a simpler and more sensitive electrochemical detection. Second, since the electrochemical oxidation of the modafinil is identified as adsorption-controlled and the SPE-CNT seen to provide an optimal electrochemical response, in section 3.2, the electrochemical detection by the AdSV technique is optimized. The accumulation time of modafinil adsorption on SPE-CNT is investigated as well as the pH of supporting electrolyte and the optimum conditions (frequency, amplitude and step potential) for determination of this analyte in 0.1 M H_2SO_4. Third, in section 3.3,

the estimation of the real surface area on SPE-CNT is studied and discussed using a Langmuir plot and the approximated theoretical area of possible molecular orientations of modafinil adsorbed on SPE-CNT. Finally, in section 3.4, the analytical performance of the SPE-CNT using AdSWV in authentic human saliva is investigated with the pre-accumulation step in undiluted samples. The electrochemical sensor is shown to selectively detect and quantify the modafinil in human saliva.

3.1. Electrochemical behavior of Modafinil

CV at a GCE was used to evaluate the solution phase electrochemical behavior of modafinil in different supporting electrolytes and different pHs. The modafnil presented an oxidation process with a well-defined peak current at +1.58 *V* (*vs* (SCE)) in H_2SO_4 solution pH 0.9, as can be seen in the Fig. 2A. In phosphate solutions (pH 2.0 to 12) this oxidation process was also observed for pH < 7.0 (Fig. S1).

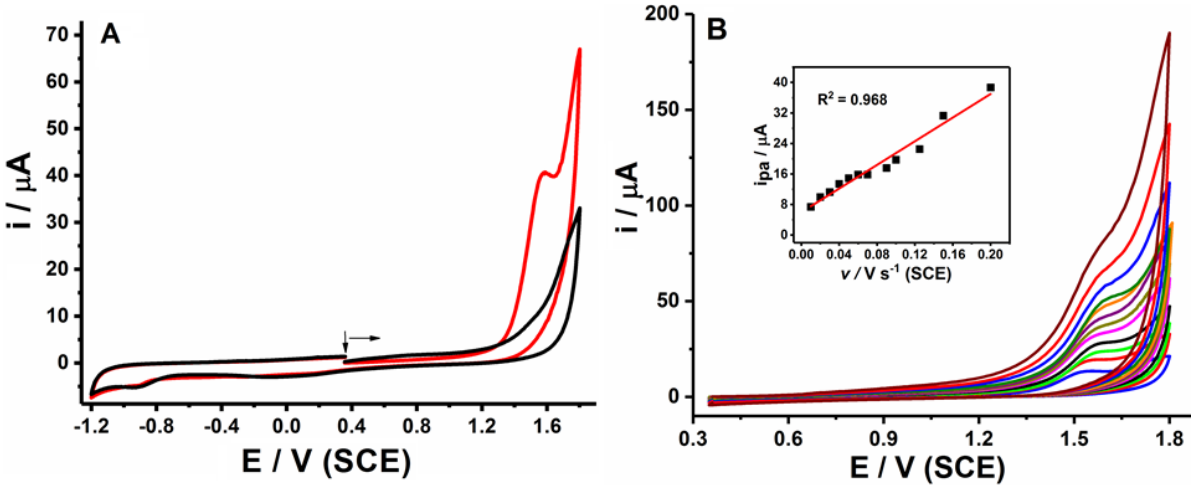


Fig. 2. (A) Voltammograms in 0.1 M H_2SO_4 at GCE (black-line) and after addition of 1.0 mM modafinil (red-line). All potential scans started at +0.35 V in the positive-going direction, see arrows. Scan rate of 50 mV s^{-1}. (**B**) Voltammograms at different scan rates (10 to 200 mV s^{-1}) for 1.0 mM modafinil in 0.1 M H_2SO_4 at GCE. Inset is plot of I_{pa} *vs v*.

Fig. 2B shows a scan rate study for the modafinil oxidation process, where the peak currents (ca. +1.*6 V* (*vs* (SCE)) were more linearly proportional to scan rate (inset Fig. 2B) with a linear regression coefficient (r^2) of 0.97 for I_{pa} (μA) = 6.0 ± 0.9 + 155.0 ± 9.0 *v* (V s^{-1}) than to the square root of the scan rate (r^2 = 0.90), indicating that this electrochemical process is at least partially adsorption-controlled at the GCE. The mass-transport control of the oxidation process of modafinil was also evaluated using a carbon microdisc as working electrode by CV in 0.1 M H_2SO_4 (Fig. 3). In these conditions, the modafinil oxidation process (ca. +1.3 *V* (*vs* (SCE)) did not show steady-state current at microelectrode in either 0.5 or 1.0 mM different concentrations (inset in Fig. 3), corroborating that this electrochemical process is adsorption-controlled.

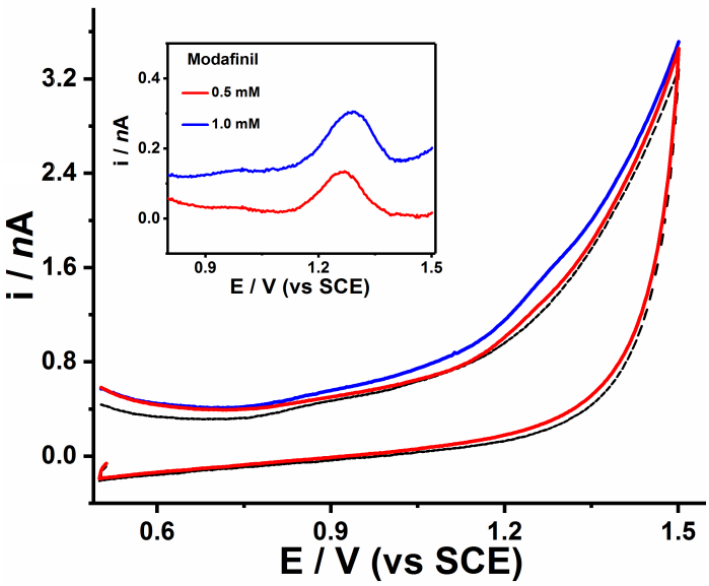
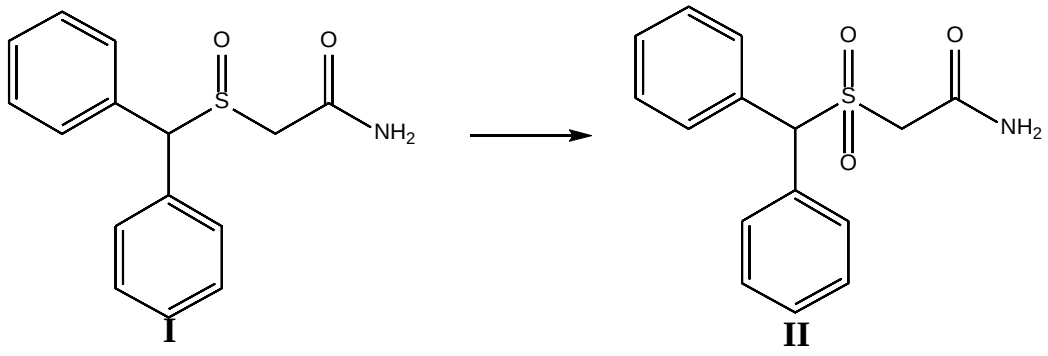


Fig. 3. Voltammograms at 10 mV s^{-1} in 0.1 M H_2SO_4 at carbon microelectrode (blank: dash-line) and after addition of 0.5mM (red-line) and 1.0 mM modafinil (blue-line). All potential scans started at +0.50 V in the positive-going direction. Inset is the background subtracted voltammograms.

The electrochemical process of the modafinil may be related to the oxidation of the sulfoxide group to sulfone group, which has been reported (scheme 2) in strong oxidation conditions when this molecule is synthesized [30], suggesting that two-electrons are transferred. In

aqueous media for pH < 7.0 at GCE, the anodic peak potential (E_{pa}) is not shifted (Fig. S1) with pH variation, which indicates that no proton is transferred in this electrochemical process, consistent with the known pKa (8.84) of the acidic proton located between the sulfoxide and amide groups of structure of modafinil [31].



Scheme2. The oxidation reaction of molecule modafinil (**I**) to modafinil sulfone (**II**) [30].

In order to facilitate the application to detection of the modafinil in saliva samples carbon SPEs were next evaluated (Fig. 4). As can be observed in Fig. 4, the SPEs also show the oxidation process for modafinil molecule, but at a slightly lower potential of 1.48 V (*vs* SCE) in 0.1 M H_2SO_4 The shift of ca. 0.1 V between the modafinil oxidation at SPEs and GCE is likely due to lower overpotential (catalytic effect) for modafinil oxidation at SPE working electrodes than at the GCE. Note that the shift of the oxidation peak potential at SPE-CNT for modafinil molecule presented below reflects the use of the printed pseudo reference electrode in the SPE.

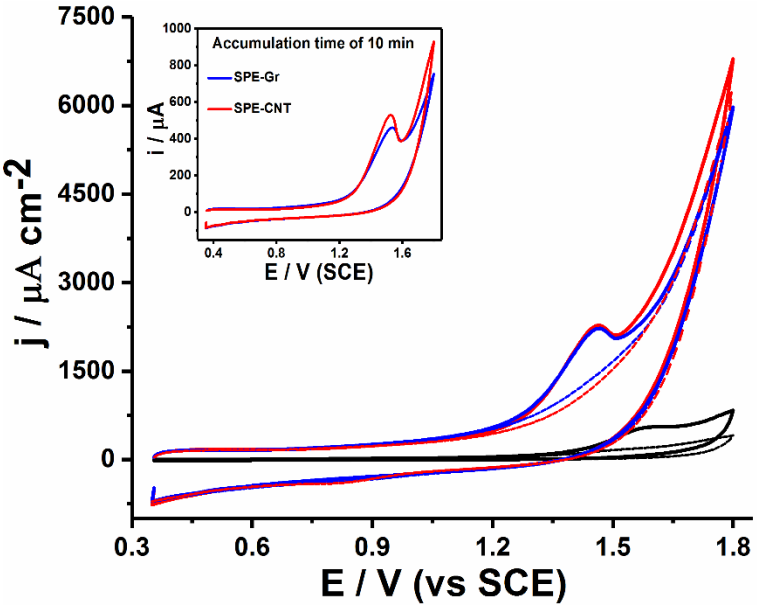


Fig. 4. Voltammograms recorded immediately after the contact of the working electrode with 0.1 M H_2SO_4 at the GCE (black lines), at the SPE-Gr (blue lines) and at the SPE-CNT (red lines). Inset is the AdSVs at SPEs with accumulation time of 10 min. All voltammograms were recorded in electrolyte before (dashed-lines) and after addition of 1.0 mM modafinil (solid-lines). All potential scans were started at + 0.35 V in the positive-going direction with a scan rate of 50 mV s^{-1}. Current densities were calculated using geometric areas of 0.068 (GCE) and 0.126 cm^2 (SPE) for the different working electrodes.

Furthermore, Fig. 4 shows that the SPEs based on carbon-nanotubes (SPE-CNT) and carbon graphite (SPE-Gr) provided a higher current density for modafinil oxidation than the GCE. It is established that carbon (graphite and nanotubes) electrodes can provide a higher sensitivity to electrochemical detection when a substance reacts via an adsorption-controlled process on the working electrode surface [32-36] such as for modafinil. The Fig. 4 also shows that the peak current for modafinil oxidation process is more sensitive at the SPE-CNT than at the SPE-Gr only when the AdSV technique is used (inset of the Fig. 4) for both SPEs after 10 min accumulation time. SPE-CNT may provide a larger true surface area for modafinil adsorption on the working electrode. In this context, the SPE-CNT was chosen for modafinil determination in authentic saliva.

3.2. Optimization of the modafinil detection by AdSV using the SPE-CNT

First, the pre-accumulation of modafinil molecules by adsorption on SPE-CNT was investigated. The effect of time was examined immediately after drop-casting (100 µL) of 1.0 mM modafinil in 0.1 M H_2SO_4 on SPE-CNT and afterwards by waiting for periods between 2.0 and 30.0 min (Fig. S2). The peak charge of the modafinil oxidation process was calculated for each experiment and a constant value was attained after 20 min (Fig. S2), indicating that the surface area of the working electrode is saturated with modafinil molecules. It is worth noting that although it is possible to detect modafinil using the AdSWV with shorter accumulation times, periods of time lower than 20 min on SPE-CNT were not sufficient for modafinil detection at less than 10.0 µM. The latter is threshold value to determinate whether human saliva contains prohibited levels of stimulant modafinil. An accumulation time of 20 min was chosen for the analytical determination of modafinil.

A pH study was also made for modafinil detection using the AdSV technique with SPE-CNT at different concentrations of H_2SO_4 (Fig. S3A) and in 0.1 M PBS solutions pH 3.0 to 8.0 (Fig. S3B) solutions, all with an accumulation time of 20 min. The electrochemical behavior of the modafinil oxidation at the SPE-CNT in different pHs was similar to that seen on GCE (Fig. 2 and S1), showing a well-defined peak current at ca. 1.35 V (*vs* Ag) in acid medium using H_2SO_4 solutions as support electrolyte (Fig. S3A) but no oxidation peak for pH > 7.0 (Fig. S3B). The concentration 0.1 M was chosen as the supporting electrolyte as this gave the largest peak current (Fig. S3A)

The AdSWV technique was optimized to give the best parameters for modafinil detection: frequency 70 Hz, amplitude 50 mV and step potential 2 mV. Standard solutions of 5.0 to 500 µM modafinil in 0.1 M H_2SO_4 were evaluated (Fig. 5). A linear relationship between the modafinil concentration and its peak charge (from the oxidation process) was obtained of 5.0 to 100.0 µmol L^{-1} (inset of Fig. 5), with r^2 of 0.997 for the following linear equation: Q (µC) = -0.12 ± 0.44 + 2.82 ±

0.08 (µA / µmol L^{-1}) [modafinil]. In these conditions, the theoretical limit of detection (LOD) of 1.2 µmol L^{-1} was obtained from $3S_B/m$, where S_B is the standard deviation (N = 10) of the background (blank) response and m is the slope of linear equation. A positive-shift of the peak potential of modafinil oxidation in higher concentration is evident in Figure 5. This can be explained by the fact that as the concentrations of analyte increases the number of adsorbed molecules on the electrode surface increases and so the peak appears at higher potentials in the linear sweep voltammogram (for electrochemically irreversible process) as the oxidation completed later in the scan. The limit of quantification (LOQ) of 4.0 µmol L^{-1} was obtained from $10S_B/m$. Note that a measurable signal of 5.0 µmol L^{-1} can be seen in Fig. 5 (red line) for modafinil determination by AdSWV at SPE-CNT.

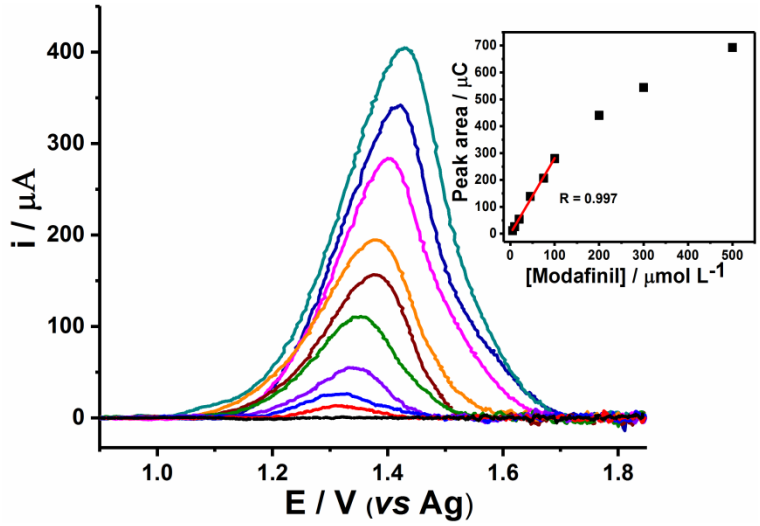


Fig. 5. AdSWVs for concentrations from 5.0 to 500 µM modafinil in 0.1 M H_2SO_4. (blank: black-line) at SPE-CNT. Amplitude 50 mV, frequency of 70 Hz and step of 2 mV. Inset is the corresponding linear regression plot.

3.3- Estimation of real surface area of the SPE-CNT

In order to characterize the adsorption of modafinil, the Langmuir isotherm was applied to the data of Fig. 5, according to following equation [37]:

$$\theta = \frac{K[C]}{1 + K[C]}$$

where θ is the fractional occupancy of the adsorption sites, K is the equilibrium constant for adsorption and [C] is the concentration of modafinil. As can be seen in Fig. 6, the Langmuir plot shows a regression linear (1/Q (μC^{-1}) = 0.00086 ± 0.00007 + 0.29 ± 0.01 1/C (μmol^{-1} L)), with r^2 of 0.998, indicating that the modafinil adsorption is well-described by the Langmuir isotherm. Using this Langmuir adsorption isotherm, the maximum charge transfer (Q_{max}) and the equilibrium constant for adsorption (*K*) were calculated to be 1,200 µC and 0.003 μM^{-1}, respectively [33].

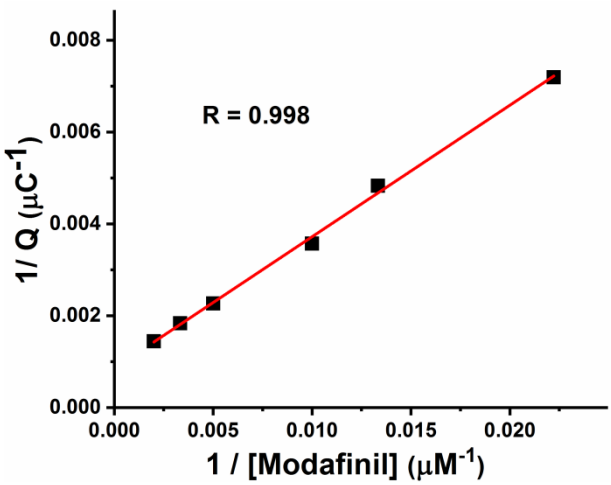
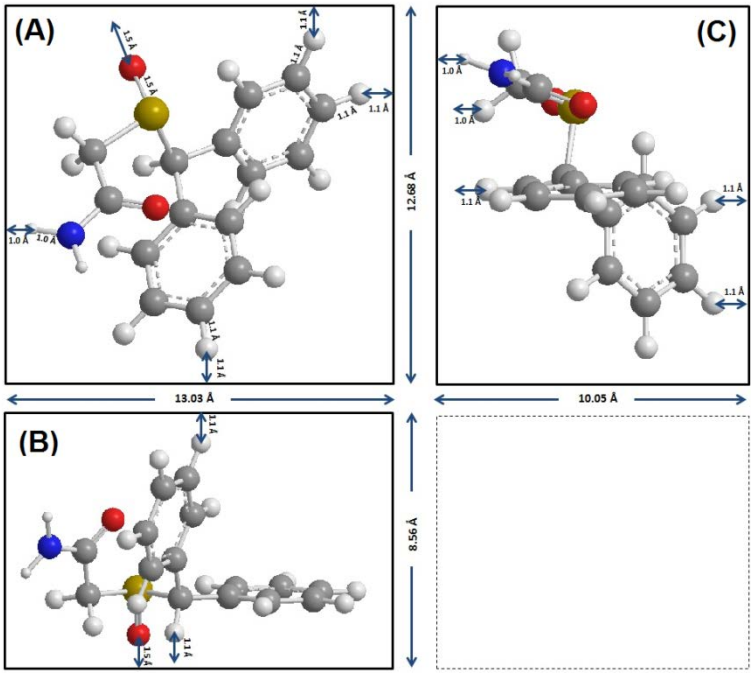


Fig. 6. Langmuir plot of 50 to 500 µM modafinil obtained from peak area shown in Fig. 5. The equilibrated surface coverage of the modafinil is measured in 20 min of accumulation time (Fig. S2).

In order to estimate the approximate area of possible molecular orientations of the modafinil adsorption on SPE-CNT, a rectangular box model was used (Scheme 3). All side lengths were estimated by trigonometry for bond lengths, bond angles and van der Waals radii of terminating atoms. Using ChemDraw 15.1 software and values tabulated by Rowland for the van der Waals radii of the terminating atoms [38]. This procedure has been reported for other organic molecules as dopamine [39], phenyl hydroquinone [40] and catechol [41]. For the modafinil

molecule, three possible adsorption orientations on SPE-CNT are shown in Scheme 3 as a flat view (A), edgewise view (B) and endwise view (C). Based on these molecule orientations for modafinil adsorption on SPE-CNT, the areas of the three sides of the "box" were calculated to be 1.7×10^{-14}, 1.1×10^{-14} and 1.3×10^{-14} cm^2, which correspond to 6.0×10^{13}, 9.0×10^{13} and 7.9×10^{13} molecules per cm^2 to flat (A_{fl}) , edgewise (A_{ed}) and endwise (A_{en}) views, respectively.

According to the value $Q_{max} = 1.2 \times 10^{-3}$ C obtained from the Langmuir isotherm (Fig. 6), the electron fundamental charge (1.602×10^{-19} C) and the number of electrons involved in the modafinil oxidation ($n = 2$), the number of molecules adsorbed on SPE-CNT (N_{Ad}) was calculated to be 3.7×10^{15} molecules. Hence, assuming one monolayer and a close packing, the real surface area available for adsorption of the porous layer of carbon nanotubes on SPE-CNT was estimated to be 42-62 cm^2 by N_{Ad} / A_{fl}, N_{Ad} / A_{ed} and N_{Ad} / A_{en}, with similar values reported for other carbon nanotube modified electrodes [32, 33]. Alternatively multilayer adsorption may occur on SPE-CNT.



Scheme 3. Rectangular box model of modafinil molecule for both **(A)** flat view, **(B)** edgewise view and **(C)** endwise view.

3.4. Detection of Modafinil in saliva samples

Modafinil detection in authentic human saliva samples by AdSV at SPE-CNT (Fig. 7), according to procedure shown in Scheme 1 was investigated. In these studies, human saliva samples were "spiked" with standard solutions of modafinil.

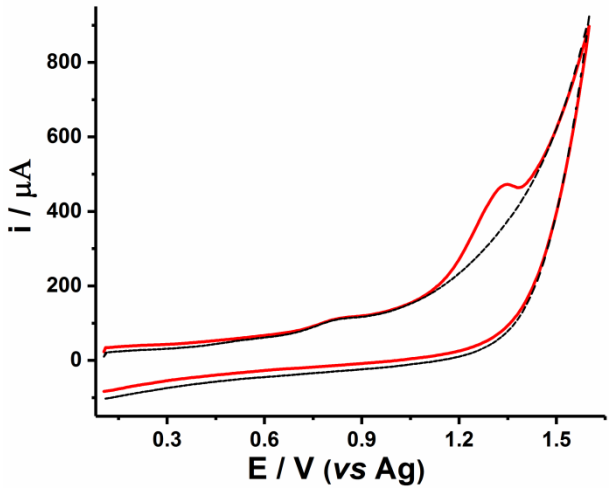


Fig. 7. AdSV after 20 min accumulation time in authentic human saliva with voltammograms recorded in 0.1 M H_2SO_4 at a SPE-CNT without (dashed black line) and with addition of 1.0 mM modafinil (solid red line). All potential scans were started at +0.1 V in the positive-going direction. Scan rate of 50 mV s^{-1}.

As can been seen in Fig. 7, the oxidation process of modafinil (ca. 1.3 V (*vs* Ag)) at SPE-CNT is clearly visible using the AdSV technique with the pre-accumulation step in authentic saliva sample and detection performed in 0.1 M H_2SO_4. Evidently, the pre-accumulation step of the AdSV technique can also be used as an extraction process of saliva samples on SPE-CNT, avoiding any need for the dilution and providing a more realistic *on-site* analysis to doping control of modafinil in authentic saliva. In these conditions, the analytical parameters for modafinil determination were investigated by AdSWV detection at SPE-CNT. The reproducibility for modafinil detection by AdSWV at SPE-CNT was examined using different electrodes to 7.5 µM and 50 µM modafinil

added in authentic saliva and the results are presented in Fig. S4. Low relative standard deviations (RSD) of 3.2 and 3.0 % for peak charges (n = 3) of the modafinil oxidation at 7.5 µM and 50 µM were obtained, respectively. This study shows that the method proposed provides a good reliability for determination of the modafinil in authentic saliva.

In order to evaluate possible interferences in saliva various common compounds (Fig. 8A) found in authentic human saliva [21, 27] was studied. As can been observed in the recorded voltammograms for ascorbic acid (red line in Fig 8A) and uric acid (green line in Fig 8A), both are oxidized at very lower potential than for modafinil oxidation. Furthermore, the added concentration of these interferents used to generate the reported voltammograms were higher than it is reported in human saliva, where the ascorbic acid and uric acid are present of ca 1.0 µM [27] and of ca. 200-300 µmol L^{-1} [27], respectively. As can also been noted in Fig 8A, the response for modafinil oxidation (blue line) was not significantly changed after addition of the investigated interferents, even at high concentrations of the uric acid (3.0 mM) and ascorbic acid (100 µM) in the human saliva sample. The proposed method provides sensitivity for modafinil (1.8 µA μM^{-1}) detection of ca. 2.0 and 100 times higher than for ascorbic acid (1.0 µA μM^{-1}) and uric acid (0.02 µA μM^{-1}), respectively. The origin of the oxidation process at ca. +0.9 V on SPE-CNT (*vs* Ag) shown in the blank saliva sample (black line in Fig. 8A) is unknown at present.

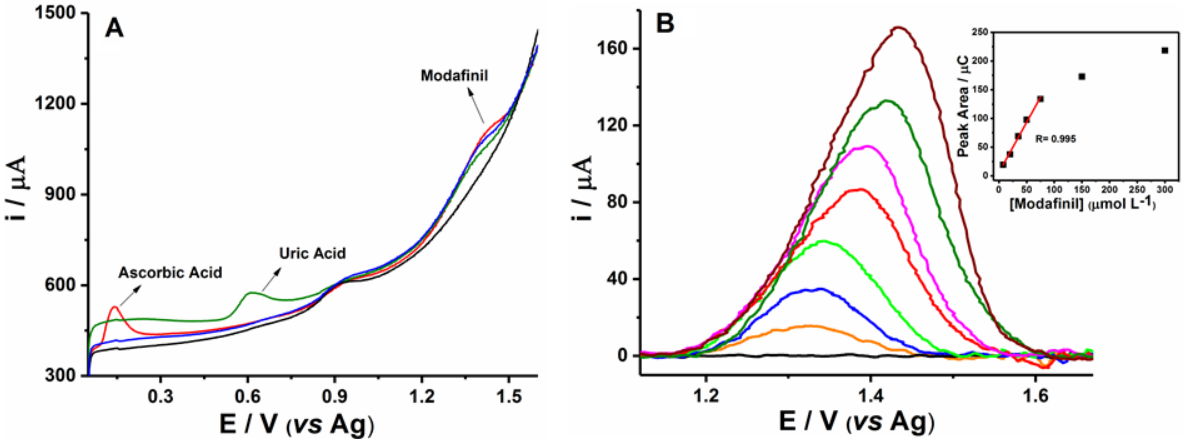


Fig. 8. (A) AdSWVs recorded in 0.1 M H_2SO_4 at SPE-CNT after accumulation time of 20 min in authentic saliva before (black line) and after addition of 50 µM modafinil (blue line) with 100 µM ascorbic acid (red line) or 3.0 mM uric acid (green line). (**B**) AdSWVs recorded (corrected background current) in 0.1 M H_2SO_4 at SPE-CNT after accumulation time of 20 min in authentic saliva (blank: black-line) with addition of 7.5 to 300 µM modafinil. Amplitude of 50 mV, frequency of 70 Hz and potential step of 2 mV

A calibration curve was evaluated for modafinil quantification in saliva samples. This study was performed by addition of modafinil standard solution in the authentic human saliva with increasing concentrations of 7.5 to 300 µM (Fig. 8B). Saliva samples (100 µL) with each concentration of modafinil were drop-casted on SPE-CNT and after 20 min the sample solution was removed using a micropipette. Next, a 0.1 M H_2SO_4 (100 µL) solution was drop-casted on SPE-CNT and the voltammogram was recorded immediately by AdSWV. The linear relationship between the modafinil concentration and its peak area (from oxidation process) was obtained of 7.5 to 75 µM, as can be seen inset in Fig. 8B, with r^2 of 0.995 for the linear equation Q (µC) = 6.19 ± 3.24 + 1.74 ± 0.07 (µC / µmol L^{-1}) [Modafinil]. The LOD of 2.0 and LOQ of 6.7 µmol L^{-1} were obtained by $3S_B/m$ *and* $10S_B/m$, respectively.

The LOD obtained by proposed method is low sufficiently to application to doping control, allowing the direct use in human saliva samples. It is worth highlighting that human saliva can contain unchanged modafinil at levels around 10.0 μM after (6-9 hours) intake of this drug [26]. A low measurable signal at SPE-CNT by AdSVW detection for 7.5 μM (orange line in Fig. 8B) shows a clear, usable response.

The present work offers advantages for modafinil determination in saliva compared to the reported method using gas chromatography with mass spectrometry detection [26], notably the simplicity of application and the low-cost for application in screening tests for doping control. Moreover, most reported papers for the detection of modafinil in other biological matrices (serum, plasma and urine) as well as pharmaceutical formulations are based on chromatographic methods [25], allowing the proposed method can be contrasted to these samples as an easier and lower-cost application.

4. Conclusions

The stimulant modafinil exhibited an oxidation process in acid media at GCE and at carbon SPEs. Studies showed that the modafinil oxidation process is adsorption-controlled at carbon electrodes and the AdSV technique can be usually used to detect this analyte in undiluted human saliva. To the best of our knowledge, for the first time, is reported an electrochemical method to detect the modafinil molecule. A simple and portable electrochemical sensor using a SPE-CNT by AdSWV is presented for a selective and sensitive (LOD of 2.0 μM) detection of modafinil in authentic saliva using a small amount (100 μL) of sample and a total assay time of ca. 30 min. Therefore, this work demonstrates an easy and inexpensive method for modafinil determination in human saliva samples using screen-printed electrodes by adsorptive voltammetry detection.

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