**A nano-carbon electrode optimized for adsorptive stripping voltammetry:
Application to detection of the stimulant selegiline in authentic saliva**

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Highlights:

- A multilayer nano-carbon modified electrode is prepared and optimized for adsorptive stripping voltammetry.
- Multilayers of Carbon Black promote adsorptive over diffusive signals.
- The electrochemical sensor is applied for the sensitive detection of the stimulant drug selegiline in authentic saliva.

Abstract: A multilayer Carbon Black (CB) modified Glassy Carbon Electrode (GCE) is proposed, developed and optimized for Adsorptive Stripping Voltammetry (AdSV). The thick (up to 1000 monolayers) multilayer CB modification offers a significant enhancement in sensitivity for AdSV but not diffusive voltammetry. The detection of the stimulant selegiline, which is a prohibited drug for athletes in sports competitions, is used as model for application of the proposed electrochemical method in saliva samples. The analytical performance of the CB-GCE for detection of selegiline in authentic saliva samples revealed a limit of detection of $0.12 \mu\text{mol L}^{-1}$ which is sufficiently low to allow a possible application of this fast method in the doping control of this drug.

Keywords: *Adsorptive Stripping Voltammetry; Carbon Black; Doping Control; Saliva; Selegiline.*

1. Introduction

1.1 Adsorptive Stripping Voltammetry and Carbon Black Modified Electrodes

Adsorptive stripping voltammetry (AdSV) is widely used to increase the range of applicability and the sensitivity of electroanalytical techniques. The method, as with stripping voltammetry in general, entails a pre-accumulation step followed by a detection step in which the accumulated material is Faradaically oxidized or reduced. In AdSV the pre-accumulation step results from the physical or chemical adsorption of the target on the electrode surface. Since the latter is often constrained to be monolayer or less it follows that the surface area of the detecting electrode is of profound importance in controlling the ultimate sensitivity of the method.

The aim of the present paper is to address the issue of developing a relatively general, widely applicable, low cost, approach to electrode modification allowing the optimization of AdSV measurements. To that end we explore the use of *thick* layers (in the range 3-30 μm) of nano-carbon (Carbon Black, CB) as a porous modification of glassy carbon electrodes (GCE). Attention is paid to the maximization of the active electrode surface area whilst retaining Faradaic control of the material adsorbed on the CB surface. The prior use of CB in Electroanalysis, and other electrode materials considered for AdSV, is summarized in the Supplementary Information of this paper.

In order to challenge our multilayer CB modified electrode we address the detection in authentic saliva of a prohibited stimulant and which is present only in trace amounts at the levels of interest to the World Anti-Doping Agency (WADA). The analysis not only demands very high sensitivity but also good reliability coupled to the use of authentic media since recent work has shown the not infrequent gross invalidity of artificial saliva as models [1].

1.2 Doping Control and Selegiline in Saliva

Due to the non-invasive collection and ease of transport and storage, saliva samples have attracted attention [1-5], notably for the detection of drugs and the diagnosis of diseases. In addition, the correlation between the intake of drugs and their excretion (either unchanged or as metabolites) in plasma, urine and saliva has been studied for many stimulants, drugs of abuse and other compounds [1, 2, 5-9]. The detection of some drugs in saliva is a viable approach to doping control.

Doping control is regulated by WADA, which publishes annually a list of drugs prohibited to be taken before or during competitions [10]. Among these drugs some stimulants are banned and these range in chemical identity from common illicit drugs (cocaine and amphetamines) to licit drugs used for treatment of Parkinson's disease [11] such as selegiline (Fig. 1). Inappropriate use of selegiline intake has been reported in 1995 in the case of an Italian boxer, who was disqualified just one day after becoming a champion [11].

Fig. 1. Molecular structure of selegiline

In general, athletes take stimulant drugs only a few hours before a sporting event because these drugs have only a short-period of activity in the human body [8]. Although it is possible to detect metabolites of these drugs in urine samples following sustained intake, the level of the non-metabolised forms is often very low and in some cases the measured metabolites concentration levels do not allow confirmation or otherwise as to whether either the drug was intentionally taken by the athlete or the intake was only used for therapeutic treatment a long time before competition [8]. Therefore, unchanged drug detection in saliva samples is potentially important to anti-doping analysis, offering the possibility of screening as well as establishing correlations between results obtained in urine and blood samples.

Although there are many methods for analysis in saliva samples, most of them are too complex for realistic *on-site* analysis for doping control, notably LC-MS and CG-MS [1]. In favourable cases, electroanalytical methods could provide portable, simpler, cost-effective and faster analysis methods than conventional procedures. In this context, electrochemical sensors are of great interest for application to saliva samples collected just hours before a sports competition. Even though electroanalytical methods have been reported for detection of some substances in saliva samples [1, 5], they have not been applied for doping control analysis. Furthermore, only very few papers have reported the electrochemical detection of selegiline but never in saliva [12-14].

In this paper, for the first time, we present a generic and simple voltammetric procedure based on AdSV using a CB nanoparticle modified electrode for selegiline determination in

authentic human saliva. The limit of detection achieved is sufficiently low to assess whether the saliva contains prohibited levels of selegiline.

2. Experimental

2.1. Chemicals and materials

All solutions were prepared with deionized water of resistivity not less than 18.2 MΩ cm (at 25 °C), obtained using a Milli-Q system (Millipore, USA). Selegiline ((2R)-N-methyl-1-phenyl-N-prop-2-ynylpropan-2-amine) hydrochloride was purchased from Sigma-Aldrich (Lancashire, UK). The potassium chloride, sodium hydroxide, sulfuric acid, monobasic and dibasic sodium phosphates were all of analytical grade and were purchased from Sigma-Aldrich (Lancashire, UK). Britton–Robinson (BR) buffer solution was prepared using boric acid (99.5% purity, Aldrich), phosphoric acid (99.99% purity, Aldrich) and acetic acid (99.5% purity, BDH) with different pHs (2 to 12). Sulfuric acid and sodium hydroxide were used to adjust the pH (2.0 to 12.3) of 0.1 M BR solution and pH (5.8 and 12.3) of 0.1 M phosphate buffer solution (PBS).

2.2. Saliva sample preparation

Authentic saliva samples (5.0 mL) were collected from healthy volunteers using Salivette® (Sarstedt, Germany) [1], according to all local guidelines for the work with human saliva. The swabs from the Salivette® were chewed by each volunteer for 1 min and then the Salivette® containing these swabs were centrifuged at 2800 rpm for 5 min. After that, saliva samples (5.0 mL) were transferred to the electrochemical cell. Note human saliva is mainly composed of water (99%), and contains also several inorganic (Na^+ , K^+ , Ca^{2+} , Cl^- , HPO_4^{2-} , etc) and organics (uric acid, creatine, etc) substances and others [1]. Synthetic saliva (Synthetic Urine e.K., Eberdingen, Germany) samples were also used, which contain only inorganic ions including Mg^{2+} , Ca^{2+} , K^+ , Cl^- , CO_3^{2-} and HPO_4^{2-} [1].

Potassium phosphates salts (monobasic and dibasic) were added to authentic saliva samples (without dilution) to adjust the pH and to tune the selectivity towards selegiline detection on carbon black modified electrodes by AdSV. The addition of salt was calculated to achieve a final concentration of 0.1 mol L⁻¹ to the phosphate buffer in the collected saliva sample. The pH measurements in authentic saliva samples were performed before (pH between 7.0 and 8.0) and

after addition of phosphates salts using a small amount (approximately 100 μL) of concentrated sodium hydroxide solution to adjust the pH to 5.8 or 12.3.

2.3. Instruments and analytical procedures

The size, shape and morphology of the CB particles used in this work was examined by transmission electron microscopy (TEM) using a Joel JEM-3000F field emission gun transmission electron microscope with an accelerating voltage of 300 kV. For TEM sample preparation, the nanoparticles were pre-dispersed in chloroform, pipetted onto a holey carbon grid (Agar Scientific, Stansted, UK), and dried under ambient conditions.

Voltammetric experiments were performed using a model $\mu\text{Autolab III}$ (Eco Chemie, the Netherlands) potentiostat controlled by NOVA software 2.0. All measurements were performed using an electrochemical cell of three electrodes in a Faraday cage thermostatted at 25 $^{\circ}\text{C}$. Prior to each measurement, pure nitrogen gas was bubbled for 5 min in all the studied solutions to remove oxygen. A graphite rod and a saturated calomel electrode, SCE (SCE +0.244 V vs. SHE, BASiInc., Japan) were used as the counter and reference electrodes, respectively. The working electrode used for this study and for the modification with carbon black material was the glassy carbon electrode (GCE, BAS Technical, USA) with a geometric area of 0.068 cm^2 .

Modifications of the GCE surface with Carbon Black (CB, M 1100, Monarch® donated by Cabot Performance, Billerica, MA, USA) were performed by drop-casting different aliquots (1 to 16 times) of 1.5 μL of a CB particle suspension (10.0 mg CB/ 1.0 mL) in chloroform (99.99% purity, Aldrich), followed by solvent evaporation for 10 min at 60 $^{\circ}\text{C}$. All suspensions of the CB were sonicated for 30 min after preparation and were used on the same day. $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$ (from Alfa Aesar, UK) and KCl solutions were used for characterization of the CB modified electrode.

Electrochemical behaviour studies were first conducted by cyclic voltammetry (CV) at a GCE in solutions of selegiline in 0.1 M BR buffer (pH 2 to 12.6) with concentrations in the range of 1.0 to 4.2 mM. Experiments using square wave voltammetry (SWV) were conducted for assessing the sensitivity for selegiline detection at GCE in synthetic saliva. The adsorptive stripping voltammetry (AdSV) and adsorptive square-wave voltammetry (AdSWV) methods for selegiline determination (range 0.1 to 100 μM) in synthetic and authentic saliva samples at CB modified GCE (CB-GCE) were studied. The amount of CB drop-casted on the GCE (1.5 to 24 μL) and the accumulation time (1.0 to 30 min) for selegiline detection in saliva samples were optimized.

3. Results and discussion

3.1. Characterization of CB modified-GCE

To characterize the particle size and morphology of CB, TEM measurements were conducted and representative TEM images are displayed in Fig. 2A, B and C. A histogram that depicts the size of the individual particles and a lognormal distribution fitting are shown in Fig. 2D. The particles appear quasi-spherical; and the size distribution analysis reveals a mean diameter of 19.3 ± 3.6 nm based on the analysis of 82 individual CB particles that are identified from the TEM micrographs, as shown in Fig. 2B.

Fig. 2. TEM images of CB particles at different spots (A, B and C). (D) Histogram that depicts the size distribution of 82 individual CB particles identified in TEM images. The mean particle size is estimated to be 19.3 ± 3.6 nm in diameter.

The area and specific capacitance of various amounts of CB drop-cast on a GCE were evaluated to investigate the CB modified electrode surfaces. The total surface area of the CB particles forming the modified electrode with different CB drop-castings was calculated from the added volume of the CB suspension drop-casted (10 mg / 1.0 mL) considering the CB nanoparticles as spheres. The mean radius of CB nanoparticles of 9.7 nm (obtained from TEM, Fig. 2) and the density for the CB suspension of 2 g/mL [15, 16] were used for all calculations. The areas obtained are shown in Table 1. The number of monolayers of CB in the modified-GCE was estimated assuming a close-packed structure with a fill factor of 91% [17], see Table 1.

In order to determine the capacitance of each CB-modified electrode, CV experiments were conducted in a solution containing 0.1 mol L⁻¹ KCl at various scan rates. As a representative example, the CVs obtained at various voltage scan rates from 10 to 800 mV s⁻¹ are shown in Fig. 3A. As can be seen, the capacitive current (at 0 V) is as expected directly proportional to the scan rate. The slope of the plot shown in the inset of Fig. 3A gives the capacitance (μF) of CB-modified electrode: $I (\mu A) = 3.5 (\pm 1.1 \times 10^{-14}) + 435.6 (\pm 2.4 \times 10^{-14}) v (V s^{-1})$. The specific capacitance (μF cm⁻²) relating to the surface area of the CB particles (not the geometric area of electrode) as a function of amount of CB drop-casted on GCE is shown in Fig. 3B and the values are listed in Table 1.

Fig. 3. (A) CVs in 0.1 M KCl solution at CB modified (6.0 μL) GCE in different scan rates (10.0 to 800.0 mV s⁻¹). Inset is the linear regression obtained of the collected current at 0.0 V vs v (V s⁻¹). (B) Specific capacitance of CB-GCE in 0.1 M KCl in function for different amounts CB (drop-casting from 3.0 to 24.0 μL), number (N) of particles of CB modified and number of monolayers of CB-modified on GCE.

Table 1: Parameters for characterization of CB modified GCE*

CB drop-casting (μL)	Total surface area of CB [†] (cm^2)	Number of Monolayers [‡]	Specific capacitance ($\mu\text{F} / \text{cm}^2$)
3.0	47.4 ± 19.0	158 ± 66	3.6 ± 1.5
3.7	58.5 ± 23.4	197 ± 83	5.2 ± 2.1
4.5	71.1 ± 28.4	237 ± 100	4.7 ± 1.8
6.0	94.8 ± 37.9	316 ± 133	4.6 ± 1.8
9.0	142.2 ± 56.8	474 ± 199	4.3 ± 1.7
10.5	165.9 ± 66.4	553 ± 232	3.3 ± 1.3
12.0	189.6 ± 75.8	632 ± 265	3.0 ± 1.2
15.0	237.0 ± 94.8	790 ± 331	2.8 ± 1.1
18.0	284.4 ± 119.4	948 ± 398	2.1 ± 0.8
21.0	331.8 ± 132.7	1106 ± 465	1.7 ± 0.7
24.0	379.2 ± 151.7	1264 ± 506	1.3 ± 0.5

[†]Total surface area of CB = $4\pi r_p^2 \times N$, where r_p is the radius of the CB particle and N is the number of particles of CB modified. [‡] Number of monolayers = $f \times N \times (\pi r_p^2) / (\pi r_{el}^2)$, where r_{el} is the geometric radius of the electrode and $f = 0.91$ (see text). *The error bar was obtained from error propagation.

According to Fig. 3 and Table 1, the specific capacitance of CB-GCE for different drop-castings was in the range of 1.3 ± 0.5 to 5.2 ± 2.1 ($\mu\text{F cm}^{-2}$) with the apparent value decreasing as the amount of CB on the electrode surface increases. For low coverages the value is close to that expected ($6.0 \pm 0.6 \mu\text{F cm}^{-2}$) [18]. The decrease with coverage is interpreted, given that thickness of the layers used of up to more than 1000 monolayers, to the progressive lack of electrical contact with particles in the outermost layers due to imperfect particle-particle contacts. It is clear that in terms of creating a maximum surface area of CB in electrical contact with the supporting GCE the number of particles that can be used is constrained but that even for surface modifications of 1000 monolayers or more useful electrical contact is possible.

Nothing that modifying layers of particles on electrode surfaces can act either to 'block' the electrode [19] or, in the case of conductive particles, provide a transition from a semi-infinite diffusive response to a 'thin layer' type signal [20], the CB modified GCE was also investigated in 0.1 M KCl solution with the addition of 1.0 mM $[\text{Ru}(\text{NH})_6]^{3+}$ as a model redox species by CV to investigate the Faradaic response of the modified electrode. Fig. 4 presents voltammograms recorded in 1.0 mM $[\text{Ru}(\text{NH})_6]^{3+}$ solution at 50 mV s^{-1} for different CB amounts drop-cast on the working electrode surface.

Fig. 4. CVs of 1.0 mM $[\text{Ru}(\text{NH}_6)^{3+}]$ solution in 0.1 M KCl at bare GCE (blue line) and at GCE modified with various amounts of CB (drop-casting of 3.0 to 24.0 μL). Scan rate: 50 mV s^{-1}

The signals in Fig. 4 show the capacitive response of the electrode as described above but with small Faradaic signals superimposed attributed to the Ru (II/III) couple with a formal potential of $-0.197 (\pm 0.002)$ V (vs SCE) in agreement with the literature [21]. It is evident that the overall response is dominated by the capacitance of the modifying CB layers. This shows that the *thick* multilayer CB modification provides a surface area much greater than that of the supporting GC electrode and hence offers a huge potential increase in sensitivity over the latter for adsorption layers but not diffusive voltammetry. The enhanced active area offers selectivity for adsorptive signals over diffusional Faradaic signals since the latter reflect the geometric area of the underlying electrode not that of the modification layers. In the light of the above insights the CB modified electrode was evaluated for the detection of selegiline in saliva samples using AdSV.

3.2. Detection of Selegiline in saliva samples

The solution phase electrochemical behavior of selegiline was evaluated using CV at an unmodified GCE in 0.1 M BR buffer solutions at pH 2.0 to 12, as shown and discussed in the Supporting Information (Fig. S1-S3). The oxidation processes of selegiline were subsequently selected for its detection in saliva samples due to the higher sensitivity than the reduction process observed (Fig. S3A). The peak currents (ca. +0.9 V (vs (SCE))) for first oxidation process of selegiline were linearly proportional to the square root of the scan rate (inset of Fig. S3B), indicating that this electrochemical process of selegiline is diffusion-controlled at the GCE.

Fig. 5A presents the CV recorded in a synthetic saliva solution with and without 1.0 mM selegiline at GCE. One clear oxidation peak at +0.88 V (vs SCE) for selegiline is evident, as well as the reduction peak at -1.0 V of the product generated by the oxidation process, according to the studies shown in the Supplementary Information (Fig. S3A).

Fig. 5. (A) Voltammograms in synthetic saliva at GCE (blank: dash-line) and after addition of 1.0 mM selegiline (red: solid-line). All potential scans started at -0.1 V in the positive-going direction, see arrows in the Fig. 5A. (B) Polynomial (order 4)-corrected curves obtained from the voltammograms recorded using SWV for concentrations from 6.2 to 62.0 μM selegiline in synthetic saliva (blank: dash-line) at GCE. Amplitude 70 mV, frequency of 70 Hz and step of 9 mV. Inset is the corresponding linear regression.

Next, SWV studies for selegiline determination were conducted in commercial synthetic saliva at a GCE. The SWV parameters were optimized for selegiline quantification in synthetic saliva and the optimum parameters for this study are frequency 70 Hz, amplitude 70 mV and step potential 9 mV. Standard solutions of selegiline in synthetic saliva were evaluated (Fig. 5B). The linear relationship between selegiline concentration and its current peak (I_{pa}) was obtained of 8.2 to 62.0 $\mu mol L^{-1}$, as can be seen from the inset of Fig. 5B, with r^2 of 0.992 for the following linear equation: $I_{pa} (\mu A) = -0.28 \pm 0.09 + 0.054 \pm 0.003 (\mu A / \mu mol L^{-1}) [\text{selegiline}]$. However, the limit of detection (LOD) obtained by linear regression is ca. 2.0 $\mu mol L^{-1}$ by SWV at GCE, which is not low sufficiently for selegiline determination in saliva samples for doping control of athletes.

Therefore, in the light of the results presented earlier CB modified electrodes were investigated to improve the performance of electrochemical sensor. Studies on GCE modified with CB (CB-GCE) were carried out for selegiline determination in saliva samples. Furthermore, to avoid any need to for the dilution of saliva samples all further experiments were exclusively performed in authentic human saliva. The accumulation of selegiline was carried out with stirring of

the solution for 20 min. Fig. 6 shows CVs in authentic human saliva for comparison of the selegiline detection at a GCE and a CB-GCE (316 monolayers CB) by the AdSV technique.

Fig. 6. Voltammograms in authentic saliva with addition of 0.1 M phosphate buffer (pH 12.3) at a bare GCE (blue lines) and at a modified (316 monolayers) carbon black (CB-GCE) electrode (red lines). The voltammograms were also recorded in saliva before (dashed-lines) and after addition of 1.0 mM selegiline (solid-lines). Accumulation time: 20 min. All potential scans were started at 0.0 V with a scan rate of 50 mV s⁻¹.

As can be observed in Fig. 6, the CB-GCE is very substantially more sensitive than a bare GCE for selegiline detection in authentic saliva with addition of 0.1 M phosphate buffer (pH 12.3), showing one well-defined oxidation peak at CB-GCE (+0.83 V *vs* SCE), which is probably an overlapping of the two oxidation processes previously verified for this drug (Fig. 3SA). On the other hand, electrochemical behavior of selegiline at the GCE (inset in Fig. 6) is similar to that observed in 0.1 mol L⁻¹ BR pH 12 (Fig. 1S), with two overlap oxidation peaks at around 0.8-1.0 V (*vs* SCE). The peak current at a bare GCE was 25 % smaller in presence of authentic saliva than in only BR buffer. The significant improvement of analytical sensitivity for the modified electrode can be attributed to the effective adsorption of selegiline on or within the CB material and/or thin layer effects [20]. The reason for adding phosphate buffer to the authentic saliva was due to the pH-dependence for selegiline detection in this type of sample. This effect is shown and discussed in the Supplementary Information (Fig. S4), indicating that the pH of the authentic saliva optimally needs to be adjusted to 12.3 for the selective determination of selegiline in saliva samples at CB-GCE, to avoid interference from uric acid.

To optimize the amount of CB on GCE for selegiline determination in authentic saliva, various coverages of CB were examined as shown in Fig. 7.

Fig. 7. (A) AdSVs for different amounts (79 to 1264 monolayers) of CB drop-casted at GCE in 1.0 mM selegiline in authentic saliva with addition of 0.1 mol L⁻¹ of phosphate buffer (to reach pH 12.3). Accumulation time: 20 min. Scan rate of 50 mV s⁻¹. (B) Plot of the corresponding number of adsorbed molecules (N) at number of monolayers of CB on GCE. N was calculated using the following equation: $N = (Q \times N_A) / (F \times v)$, where Q is the charge (from peak integration) passing during selegiline oxidation taking into account the baseline subtraction using a polynomial function (order 4), N_A is Avogadro's number, F is the Faraday constant and v is the voltage scan rate.

As can be observed in Fig. 7A, the larger the amount of CB drop-casted on GCE (from 79 to 1264 monolayers) the higher is the current signal observed for selegiline oxidation. The number of adsorbed selegiline molecules (N) on CB-GCE was calculated from the area under the peak of selegiline oxidation (Fig. 7A), and is displayed in Fig. 7B, showing a limiting benefit of modification above ca. 800 monolayers, consistent with the results presented above for the capacitance of the CB layers.

The achieved high capacity for the adsorption of selegiline molecules on CB-GCE in authentic human saliva provides a good sensitivity for the detection of this drug. It is evident from the data in Fig. 3B and Table 1 that *thick* layers of adsorbing material are helpful in promoting sensitivity especially in comparison with the unmodified electrode. 790 monolayers of CB drop-casted on GCE was selected for selegiline determination in saliva samples corresponding to the limit of Fig. 7B. The AdSWV parameters for the modified electrode were optimized and the given values of: amplitude of 30 mV, frequency of 10 Hz and step potential of 5 mV. The reproducibility of the modification with CB drop-casting of 15 μL (790 monolayers) into GCE was examined for detection of 100 μM selegiline in authentic saliva by AdSWV and the results are presented in Fig. S5A. Low relative standard deviations (RSD) of 2.0 % for current peak and 2.8 % for peak area (n = 3) of analyte were obtained. The effect of accumulation time of selegiline on CB-GCE was

investigated for determination of the low concentration of 10.0 μM in the authentic saliva (Fig. S5B) by AdSWV. In these conditions, the accumulation times of 20 and 30 min on CB-GCE showed similar peak currents and areas (Fig. S5B). An accumulation time of 20 min was chosen for analysis in saliva of this drug. We noted that accumulation times lower than 20 min on CB-GCE were not sufficient for selegiline detection at 0.5 μM .

3.3 Analytical parameters for detection of selegiline in authentic saliva

After all optimizations, the electrochemical sensor based on CB-GCE by AdSWV was evaluated by addition of selegiline standard solution in the authentic human saliva with increasing concentrations of 0.5 to 66.0 μM (Fig. 8A). The linear relationship between selegiline concentration and its current peak was only obtained in low concentrations, as can be seen in Fig. 8B, with r^2 of 0.992 for the following linear equation: $I_{pa} (\mu\text{A}) = 14.8 \pm 1.6 + 10.8 \pm 0.28 (\mu\text{A} / \mu\text{mol L}^{-1}) [\text{selegiline}]$. Table 2 summarizes linear ranges, sensitivity and LODs for selegiline determination in saliva at bare GCE and CB-GCE using SWV and AdSWV, respectively.

Fig. 8. (A) Polynomial (order 4)-corrected curves obtained from the voltammograms recorded using AdSWV (shown in Fig. S6 in the Supplementary Information) for concentrations from 0.5 to 66.0 μM selegiline in authentic saliva (blank: dash-line) at CB-GCE after accumulation time of 20 min. Amplitude 30 mV, frequency of 10 Hz and step of 5 mV. Inset is a zoom-in of the lowest measurable signal, corresponding to a concentration of 0.5 μM . (B) The plot of peak currents from the corresponding curves in Fig. 8A; inset is the linear regression obtained at lower concentration range. The measurements were performed in triplicate and the error bars were smaller than the symbol presented in Fig 8B.

Table 2: Analytical parameters for selegiline determination in saliva by GCE and CB-GCE.

Working Electrode	Voltammetric technique	Linear Range ($\mu\text{mol L}^{-1}$)	Sensitivity ($\mu\text{A}/\mu\text{mol L}^{-1}$)	LOD* ($\mu\text{mol L}^{-1}$)	Saliva Sample
GCE	SWV	8.2 - 62.0	0.05	1.86	Synthetic
CB-GCE	AdSWV	0.5 - 10.0	14.7	0.07	Synthetic
CB-GCE	AdSWV	0.5 - 10.0	10.8	0.12	Authentic

* $3 \times S_B / b$; S_B : standard deviation of background response, $n = 10$; b : sensitivity.

The comparison (Table 2) between the modified and unmodified electrode for selegiline determination in synthetic saliva shows the high sensitivity of the modified electrode (294 times higher) and a LOD of 27 times lower than the bare GCE. We note that the analytical parameters for selegiline determination using other electrochemical sensors are not compared with our method because previous sensors were not applied to saliva samples.

It is reported that the human saliva can contain unchanged selegiline at levels around 50 ng mL^{-1} or 0.30 $\mu\text{mol L}^{-1}$ after (2-4 hours) intake of this drug and, of 150 ng mL^{-1} (or 1.0 $\mu\text{mol L}^{-1}$) of its main metabolite (methamphetamine) [8]. At this low level of selegiline (0.5 $\mu\text{mol L}^{-1}$) there is a measurable signal at CB-GCE as can be noticed from the inset of Fig. 8A. Therefore, the LOD for selegiline (0.12 $\mu\text{mol L}^{-1}$) at the CB-GCE allows direct use in authentic saliva for example in doping control of selegiline.

4. Conclusions

An electrochemical sensor based on CB-GCE exhibited a high capacity for selegiline molecules, allowing the sensitive detection of this analyte by AdSWV (LOD of 0.12 μM) in human saliva. Studying the amount of CB at GCE on the behavior of the electrode showed that the number of monolayers of CB which can be usefully used to modify the electrode was surprisingly high but is ultimately probably limited by the loss of electrical contact of the outermost layers. Therefore, this work demonstrates an alternative, simple and inexpensive method for selegiline determination in human saliva samples using CB-GCE by adsorptive voltammetry detection.

5. Acknowledgements

W.T.P dos Santos wishes to thank CNPq (Grant No. 203720/2017-2) for a post-doctoral grant and FAPEMIG (Grant No. APQ-03637-16) for financial support. H. M.A. Amin gratefully acknowledges the German Research Foundation DFG for funding (Grant No. AB 702/1-1).

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