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Electrochemical determination of nicotine in smokers' sweat

Eda Mehmeti[a,*], Tugba Kilic[a], Clement Laur[b], Sandro Carrara[a]

[a] École Polytechnique Fédérale de Lausanne (EPFL), Integrated Systems Laboratory (LSI), 1015 Lausanne, Switzerland
[b] Centre de Recherche et Développement Pierre Fabre, Toulouse, France

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ABSTRACT

In this work, we report the demonstration of the capability of electrochemical sensors to provide measurements directly on the sweat of volunteers to successfully discriminate heavy smokers by light ones. Since its importance as pharmaceutical treatment-agent for nicotine replacement therapy for purposes of tobacco cessation, and since that is the key compound for the development of reduced-risk cigarettes by tobacco industry, nicotine is one of those markers that are of interest for monitoring in human sweat. Especially in therapies for purposes of smoking cessation, easy methods for an easy measure of nicotine on the patients is highly required for a fine adjustment of the nicotine dosage as release, e.g., by through therapeutic patches. Therefore, we have checked the use of a simple, inexpensive and very sensitive electrochemical sensor for quantification of nicotine in smokers' sweat. Proposed sensor is capable for fast, label-free and pretreatment-free detection of nicotine in human sweat as collected directly from the skin of heavy and light smokers. Our finding demonstrates as we can detect nicotine from human skin with sweat-collecting patches thanks to its lower detection limit (0.59 μM) as compared to the physiological limit of nicotine in human blood (12.33 μM), while keeping under control the selectivity with respect various interfering compounds find in sweat too.

1. Introduction

Nicotine is the main alkaloid found in tobacco leaves, accounting for about 95% of the total alkaloid content, which are used in the production of cigarettes, cigars or flake tobaccos with content varying from 1 to 30 mg/g [1].

Currently, more than 1.2 billion people worldwide consume different tobacco products that results in nicotine addiction [2]. The regular intake of nicotine through smoke of the burning tobacco products is toxic for both active and passive smokers and can cause several negative outcomes in human health such as cardiovascular, respiratory, central nervous diseases and even cancer [3]. This enormous consumption of tobacco products worldwide leads to around 4.9 million deaths per year. Absorption of nicotine can be through the oral intake, lungs, urinary bladder, gastrointestinal tract, and its base form can be also easily absorbed through the skin, and can cause a poisoning in a contact with nicotine containing pesticides too [4]. Therefore, several products are proposed as a replacement therapy to avoid tobacco consumption and to quit smoking. Such products include nicotine in the form of a nicotine chewing gums, nicotine patches, and nicotine tablets [5]. As it is evidenced by many studies, nicotine's fatal dose in adults was specified to be 60 mg that corresponds to nicotine concentration of 2 mg/L (12.33 μM) in blood and 4 mg/L (24.66 μM) in plasma [6] In addition to that, various studies have been performed for the analysis of nicotine and cotinine in different physiological fluids rather than blood and plasma but in urine, sweat and saliva [7] with liquid chromatography, tandem mass spectroscopy [8] colorimetry and immunoassay methods [9]. According to the results of those studies, the concentration value in sweat for cotinine was 21.4 – 202 ng/patch, nicotine was 150 – 2498 ng/patch [7] while those values were vastly different in different body fluids, for instance the cotinine concentrations were found to be 1.5, 1.7 and 5 μg/L in serum, saliva, and urine respectively with 280-fold increase in urine and 180-fold increase in plasma [10].

However, the physiology and metabolism of each smoker is different and therefore, personalized dosing of nicotine replacement is of great importance for designing the products that will respond to with the same efficiency at each individual. Therefore, critical monitoring and determination of nicotine is of paramount importance in fields of medicine, toxicology and tobacco industry, and necessitates the development of simple, accurate, sensitive, and selective analytical methods [11]. Despite of the numerous methods based on gas chromatography [12–14], high performance liquid chromatography [15,16], capillary electrophoresis [17,18], spectrophotometry [19] and radioimmunoassay [20] which have been developed so far, none of

* Corresponding author at: Karl-Franzens University of Graz, Institute of Chemistry/Analytical Chemistry, Universitaetsplatz I/I, 8010 Graz, Austria..
E-mail address: eda.mehmeti@uni-graz.at (E. Mehmeti).

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them meet the requirements of the current market based on tobacco consumption for the quantification of nicotine in biofluids since those methods require time-consuming sample-preparation steps, expensive instrumentation, and highly skilled workers.

Although electrochemical methods are advantageous thanks to their inexpensive, simple, robust, sensitive and fast detection schemes [21,22], and in addition, it is difficult to design a sensor for nicotine detection since, majority of working electrodes with different potential windows than that of nicotine are not suitable for nicotine detection. Moreover, the interference of other substances might prevent selective detection of the nicotine.

As one of the biofluids, sweat is a good candidate not only for enabling non-invasive monitoring of biomarkers but also for drug intake management for verification of drug abuse as well as drug-dosage personalization where sweat collecting patches are used for the purpose of sweat collection from volunteers [23]. As being the only validated method for sweat collection, those patches are composed of an absorbent pad where the sweat is concentrated, an acrylate adhesive layer for making patch wearable on skin, and a thin transparent polyurethane film to isolate the patch by the environment.

In addition to chromatographic and spectrophotometric methods, sweat monitoring via electrochemical sensors/biosensors is also a very active research area of medical diagnostics with thrilling examples of glucose [24], lactate [25], pH [26], drug [27] and ion [28] detection. In Table 1 several electrochemical methods are listed which were reported so far for the detection of nicotine inside different buffers. Up to now, there is not any sensor developed for the quantification of nicotine in human sweat of heavy and light smokers.

In this work, we report for the first time to the best of our knowledge, a simple electrochemical sensor to quantify nicotine levels in sweats of heavy and light cigarette smokers. The novelty of the work is in simplicity of the sensor design including fast, mediator- and pretreatment-free features for the sweat analysis that is based on off-shelf commercially available parts, i.e, screen printed electrodes and sweat collecting patches. The limit of detection of the sensor is (LOD) 0.59 μM or 191.42 ng/patch that is lower than the toxic concentration of nicotine in blood (12.33 μM) and in the physiological range of nicotine in sweat 150 – 2498 ng/patch [7].

2. Materials and methods

2.1. Chemicals and solutions

Nicotine from Acros OrganicsTM (purity ≥ 99%) was obtained from Fisher Scientific. Ascorbic acid, uric acid, dopamine hydrochloride, cotinine, glucose, glutamic acid, pyruvic acid, calcium chloride, magnesium chloride, zinc nitrate hexahydrate, lead(II) nitrate, ammonium chloride, lactic acid, urea, glacial acetic acid, sodium chloride, sodium dihydrogen phosphate, disodium hydrogen phosphate and sodium hydroxide were purchased from Sigma-Aldrich (Switzerland).

DropSense screen-printed carbon electrodes (DRP-C110, 75 units/pack), were purchased from Metrohm AG (Switzerland) and used by dipping them into the measurement solution. Working (4 mm diameter) and counter electrodes were made of carbon and reference electrode was made of silver/silver chloride.

Ultra pure water with a resistivity of 18.2 MΩ cm (Millipore Milli-Q system) was used throughout this work. Phosphate buffer solution (PBS, 0.1 M, pH 7.4) was used as supporting electrolyte. For the pH adjustment either hydrochloric acid or sodium hydroxide solutions were used. Stock solution of nicotine (10 mM; 1 mM) was prepared by dissolving corresponding amounts of the analyte in water and stored at 4 °C in the dark. The working and calibration solutions were prepared from the stock solution immediately just before measurement by appropriate dilution with supporting electrolyte.

Artificial sweat solution was prepared according to a recipe described elsewhere [29]. Briefly, 327 mmol/L ammonium chloride, 166 mM lactic acid, 83 mM urea, 42 mM acetic acid, 34 mM sodium chloride in deionized water and adjusting the pH to 7.4 by addition of 2 M sodium hydroxide. All chemicals used in this work were of analytical grade and used without any further purification.

2.2. Instrumentation

Cyclic voltammetric and differential pulse voltammetric measurements were performed using a potentiostat/galvanostat (Autolab PGSTAT101) controlled by a computer with the corresponding software (Nova 1.10.5). The electrochemical cell was equipped with unmodified screen-printed carbon electrode. All of the pH values were measured using a pH meter (model pHenomenal pH 1000 L, VWR) with a combined electrode (glass-reference electrode) that was calibrated every day by using standard buffer solutions. All potentials given in the text

Table 1
Comparison of proposed method with some previously reported electrochemical methods for electrochemical determination of nicotine.

Electrode	Electrolyte	Method	Linearity (μM)	LOD (μM)	Sample	Ref.
CPE	PBS, pH 7.5, BRBS pH 11	SWV	50–500, 50–1000	6.1, 3.2	cigarettes liquid	[1]
TiO_2/PEDOT/ITOE	PBS, pH 7.4	AMP	0–5000	4.9	–	[2]
PGE	PBS, pH 7.0 + SDS 2 mM	SWV	7.6–107.5	2	cigarettes	[3]
BDDE	BRBS, pH 8	SWV	5–500	3.1	cigarettes	[30]
BDDE	BRBS, pH 8	DPV	0.5–202.5	0.3	cigarettes, cigar, pharmaceuticals	[31]
p-AHNSA/GCE	PBS, pH 7.5	SWV	1–200	0.9	cigarettes	[32]
NGS-SPCE/RGO-SPCE/GO-SPCE/	PBS (pH 7.4)	CV	0–200	0.05, 0.08 and 0.27 μM	Cigarettes, artificial urine	[33]
EA/GCE	PBS, pH 7	SWV	1–200	0.7	cigarettes	[34]
MWCNT/GCE	$Na_2C_2O_4$, pH 4.5	DPV	31–1900	9.3	cigarettes	[35]
RGO/DPA/PGE	$Na_2C_2O_4$, pH 4.5	DPV	31–1900	7.6	cigarettes, cigar	[36]
MWCNT/GCE NCB/GCE	PBS, pH 7.4	CV	10–50, 2–10	5, 2	–	[37]
CNC/SPCE	PBS, pH 7.4	SWV	10–1000	2	saliva	[38]
MWCNT/ACS/GCE	PBS, pH 8	AMP	–	1.4	–	[39]
$CuWO_4$/rGO/Nf/GCE	PBS, pH 7	AMP	0.1–0.9	0.04	cigarettes, urine	[40]
SPCE	PBS, pH 7.4	DPV	1–375	0.6	sweat	This work

Abbreviations: BDDE: Boron-doped diamond electrode; BRBS: Briton Robinson buffer solution; SWV: Square wave voltammetry; DPV: Differential pulse voltammetry; MWCNT: Multi-walled carbon nanotubes; ACS: alumina-coated silica; PBS: Phosphate buffer solution; AMP: Amperometry; p-AHNSA: poly(4-amino-3-hydroxynaphthalene sulfonic acid); NCB: Nano carbon black; EA: Electrochemically activated; CPE: Carbon paste electrode; SDS: Sodium dodecyl sulfate; RGO: Reduced graphene oxide; DPA: (E)-1-(4-((4-(phenylamino)phenyl)diazenyl) phenyl)ethanon; PGE: Pencil graphite electrode; PEDOT: poly(3,4-ethylenedioxythiophene); ITOE: Indium/tin oxide electrode; CNC: Carbon nanotube cluster; SPCE: Screen-printed carbon electrode; GCE: Glassy carbon electrode; Nf: Nafion; $CuWO_4$: copper tungstate; NGS: nitrogen-doped graphene sheets.

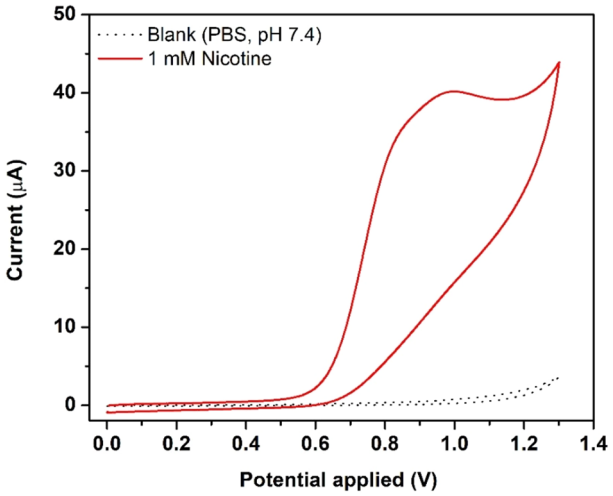

Fig. 1. Cyclic voltammograms obtained at the unmodified SPCE in the absence (Blank; dashed-line, black color) and presence of 1 mM nicotine (full-line, red color) in 0.1 M PBS (pH 7.4) at a scan rate of 100 mV s^{-1}.

are versus the pseudo reference electrode at room temperature.

2.3. Measurement procedures

Cyclic voltammetry (CV) with a scan rate of 100 mV s^{-1} was used for characterizing the electrochemical behavior of nicotine at the unmodified electrode surface. The investigated solutions were transferred into the voltammetric cell and the voltammograms (usually 5 cycles) were recorded in a potential range between 0 V and + 1.3 V after addition of particular concentrations of nicotine.

DPV parameters were optimized using a concentration of 1 mM nicotine in the measurement solution in a potential range between 0 V and + 1.3 V and the method was applied for further quantification of nicotine (pulse amplitude of 0.14 V and pulse time 0.04 s).

The calibration curves were evaluated from the peak currents of analyte representing the average value from three successive additions of standard solutions of nicotine.

2.4. Interference studies

Oxidation behavior of the most possible interfering compounds which may occur in sweat samples was analyzed including common ions: Ca^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}; organic compounds: glucose (GLU), ascorbic acid (AA), uric acid (UA), pyruvic acid (PA), and glutamic acid (GA); and cotinine (COT) as the main metabolite of nicotine (NIC). These compounds were analyzed by adding them to the artificial sweat solution (pH 7.4) containing 20 μM in 1-fold mass ratio of NIC (1:1), and the changes in the peak current of 20 μM NIC were compared in the presence of selected interfering compounds. It was considered that tested compounds strongly interfere with the determination of nicotine if they give signal changes more than 10%.

2.5. Artificial sweat solution

Multiple additions of standard solution of nicotine in the concentrations 10, 100, 200 and 300 μM were made into the artificial sweat solution and the voltammetric responses were monitored using unmodified SPCE and DPV under optimized experimental conditions.

2.6. Human sweat collection and extraction

Commercially available PharmCheck® Sweat Patches were bought from the PharmChem, Inc. consisting of a special absorbent pad where the sweat is collected. Sweat patches were applied to two apparently smoker volunteers on the measurement day, labelled as heavy (30 cigarettes/day) and light smoker (10 cigarettes/day). Sweat patches were applied to volunteers according to manufacturer's protocol. Briefly, the volunteer's upper arm area, was cleaned with alcohol wipes before patch application. The volunteers performed strong physical exercises for about 1 h. Then the patches were removed, placed in a 50 mL tube and analyzed immediately on the same day. Therefore, nicotine was extracted from the whole patch by adding 5 mL of methanol to the 50 mL tube, followed by shaking for 30 min. Then, the methanol extract was removed quantitatively and evaporated to dryness [29]. The residue was dissolved in 2 mL of PBS 0.1 M (pH 7.4) and transferred to electrochemical cell for the measurements. Afterwards, recovery experiments were done by spiking different concentrations of nicotine. All experiments were performed three times, figure of merits for the sensing performance estimated from calibration curve, and given as mean value.

3. Results and discussion

3.1. Electrochemical behavior of nicotine on unmodified SPCE

Cyclic voltammetry was employed to study electrochemical behavior of NIC on unmodified SPCE. All necessary factors, which may influence to the current response of NIC were carefully studied to determine the best conditions at which the best analytical performance can be achieved. Fig. 1 shows the corresponding voltammograms in the absence (Blank, PBS, pH 7.4) and presence of 1 mM NIC in PBS (pH 7.4) on unmodified SPCE. In accordance with many other investigations during anodic scan NIC undergoes oxidation at the surface of the unmodified SPCE starting at a potential of around + 0.6 V, and showing a high oxidation peak currents compared to the blank when no NIC was added. During a cathodic scan, by reversing at + 1.3 V, no voltammetric response which may correspond to the reduction of NIC was observed. Thus, we conclude that the oxidation of NIC is irreversible at unmodified SPCE as supported by previously reported data [30,31]. Additionally, the NIC oxidation potentials in our study, at unmodified SPCE are much lower compared to the unmodified boron-doped diamond electrodes (+1.3 V and + 1.45 V) [30,31] suggesting a faster electron transfer rate. The obtained background current was satisfactorily low, showing the benefits of the unmodified SPCE as a sensing platform.

3.2. Effect of pH of supporting electrolyte

Nicotine's two pKa values of 3.12 and 8.02 correspond to the monoprotonated and deprotonated form present in the molecule depending on the pH of the media. In order to optimize the pH value of the supporting electrolyte for the electrochemical oxidation of NIC at the unmodified SPCE, the effect of pH on the peak current (Ip) and the peak potential (Ep) was investigated using CV in the pH range from 5.0 to 8.0 with 0.5 pH unit increments; and additionally, the pH value of 7.4, which matches the physiological pH, was tested. When pH values were adjusted to lower than 5.0, the current responses of NIC were not detectable (data not shown). CVs were recorded in 0.1 M PBS in the presence of 1 mM NIC and the results from the plot of Ep and Ip values as a function of pH are illustrated in Fig. 2. The electrochemical behavior of NIC at unmodified SPCE over the tested pH range yielded an irreversible oxidation process. The obtained results indicate an increase of the oxidation peak current of NIC when increasing the pH value of the supporting electrolyte with a maximum observed at pH 7.4 showing that the oxidation process is affected by pH value. At pH values above 7.4 oxidation peak was split in two overlapping peaks which is in agreement with the previous reports [32]. However, the oxidation peak potentials of NIC shifted towards less positive values with the increase of the pH values, indicating proton participation in the electrode

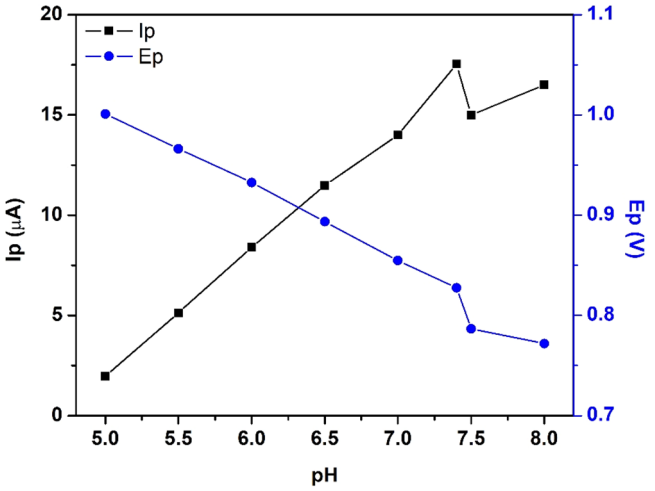

Fig. 2. Effect of the pH value on the peak potential (○) and peak current (□) of 1 mM nicotine at the unmodified SPCE in 0.1 M PBS (pH 5.0–8.0) using cyclic voltammetry at a scan rate of 100 mV s^{-1}.

reaction. Ep is linearly pH dependent in the range from 5.0 to 7.4 and can be expressed with Eq. (1):

$$Ep(V) = -0.073 \times pH + 1.368 \ (R^2 = 0.999) \quad (1)$$

The slope value indicates that the number of electrons and protons involved in the electrode reaction is equal, which is in agreement with previously reported data [28–30]. The mechanism for the electrochemical oxidation of NIC was speculated by Suffredini et al. using BDDE in alkaline pH of supporting electrolyte [30]. Therefore, the pH 7.4 was selected as the most suitable and optimum pH value in order to maintain a stable nicotine form during the quantification.

3.3. Effect of scan rate

In order to evaluate the nature of the electrochemical reaction on unmodified SPCE, the effect of the scan rate (ν) on the oxidation peak current and peak potential of NIC was examined in the ν range from 10 to 300 mV s^{-1} using CV in PBS at pH 7.4 (Fig. 3A). With increase of a scan rate, the peak potential for the oxidation of NIC slightly shifted to more positive values without any cathodic peak current, confirming an irreversible electrochemical reaction. The linear increase of peak current (I_p) of NIC with respect to the increase of the square root of the scan rate ($\nu^{1/2}$) expressed by Eq.2 suggests diffusion-controlled oxidation process (Fig. 3B).

$$Ip(\mu A) = 1.128 \times \nu^{1/2} + 6.438 \ (R^2 = 0.998) \quad (2)$$

3.4. Optimization of DPV parameters

Thanks to the low background currents and resulting lower detection limits achieved, we chose DPV for for the quantification of NIC. The most important experimental parameters of the method (pulse amplitude and pulse time) were optimized since they may affect the analytical response of the analyte. Experiments were carried out in PBS (pH 7.4) in presence of 1 mM NIC in the way that when one investigated parameter was varied the other was kept in a fixed value. Pulse amplitude was tested in the range from 10 to 200 mV (with the pulse time fixed at 25 ms). When amplitude was increasing the oxidation peak current of NIC was also increasing with concomitant widening of the peaks. Thus, pulse amplitude of 140 mV was selected as a compromised most appropriate value, with respect to the current response and peak shape of NIC. Moreover, several experiments done by keeping the pulse amplitude fixed at 140 mV but varying the pulse time in the range from 10 to 70 ms returned the value of 20 ms as the optimum, with the highest peak current and stable peak shape. Therefore, these optimized experimental parameters were employed in all next experiments.

3.5. Analytical performance of the sensor

The analytical performance of the sensor was determined by differential pulse voltammograms recorded at optimized experimental parameters for different NIC concentrations in PBS (pH 7.4) at the unmodified SPCE. The results are presented via differential pulse voltammograms (Fig. 4A) and calibration curve (Fig. 4B) drawn via average Ip values (n = 3) at corresponding NIC concentrations in the range of 1–375 μM. As it is clearly seen, the oxidation peak current of NIC is linearly proportional with NIC concentration and the numerical expression for the regression line in the calibration curve (Eq.3) shows a very good fit as seen by R^2 value:

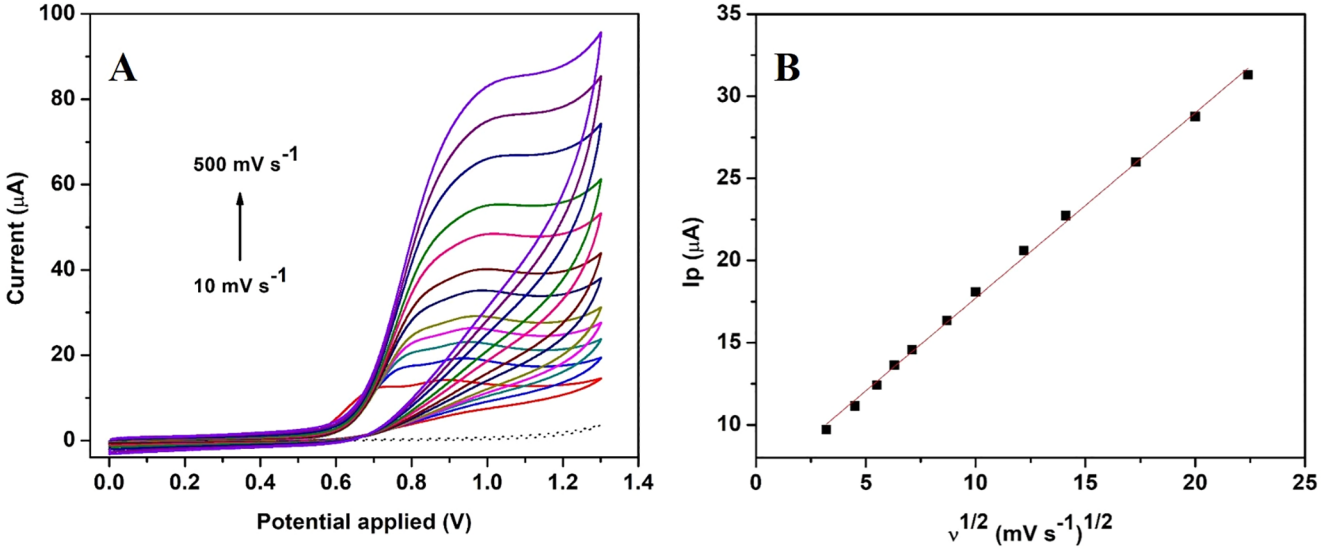

Fig. 3. A) Cyclic voltammograms obtained at the unmodified SPCE in the absence (Blank; dashed-line, black color) and presence of 1 mM nicotine in 0.1 M PBS (pH 7.4) at different scan rates from 10 to 300 mV s^{-1}. B) Ip as a function of $\nu^{1/2}$ for the oxidation peak of NIC.

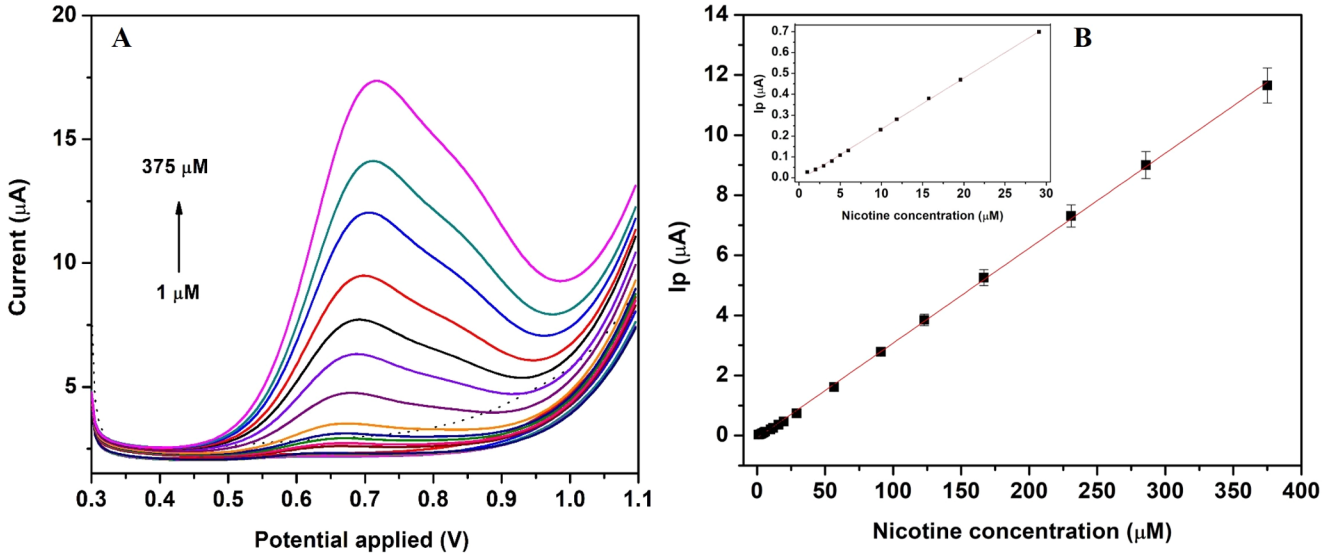

Fig. 4. A) Differential pulse voltammograms obtained at the unmodified SPCE in the absence (Blank; dashed-line, black color) and presence of different concentrations of nicotine (1–375 μM) in 0.1 M PBS (pH 7.4) at pulse amplitude of 140 mV, pulse time 20 ms and scan rate of 50 mV s^{-1}. B) Ip as a function of NIC concentration including the error bars.

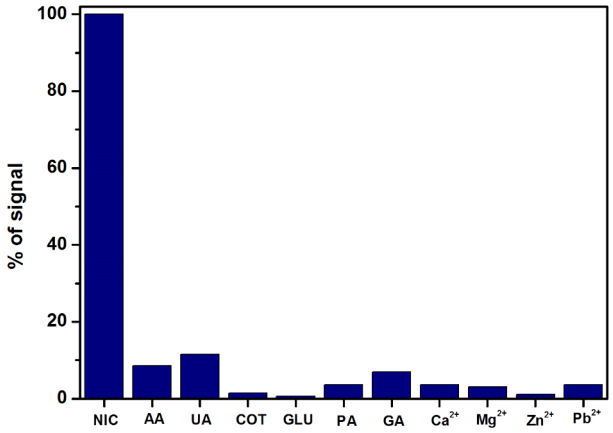

Fig. 5. Signals of tested interfering compounds in the same concentration with nicotine (20 μM) expressed as relative signals of nicotine at the unmodified SPCE in 0.1 M PBS (pH 7.4) at pulse amplitude of 140 mV, pulse time 20 ms and scan rate of 50 mV s^{-1}.

$$Ip(\mu A) = 0.0316\ (\pm 0.0002) \times c\ (\mu M) - 0.0795\ (\pm 0.0205)\ (R^2 = 0.999) \quad (3)$$

LOD (3σ-value) and limit of quantification (LOQ, 10σ-value) were estimated from the calibration curve to be 0.59 μM and 1.97 μM NIC, respectively. Compared to other electrochemical approaches that employ various electrode architectures with nanomaterials and/or polymers as listed in Table 1, our proposed unmodified SPCE sensor shows significantly lower limits of detection without any requirement for a pre-treatment step. In addition to the low LOD, the detection range is broader than most of the sensors shown in Table 1 and represnts the first kind of electrochemical sensors that detects NIC in sweat since others only quantify NIC in cigarettes, cigar, urine and saliva. X. Li et al, reported SPCE sensor modified with graphene derivatives, namely nitrogen-doped graphene sheets-SPCE (NGS-SPCE), reduced graphene oxide-SPCE (RGO-SPCE) and graphene oxide-SPCE (GO-SPCE) of which LOD values were 0.05, 0.08 and 0.27 μM, respectively. The LOD value of our sensor, 0.6 μM is closer to the one for GO-SPCE in that study and higher than NGS-SPCE and RGO-SPCE due to the fact that graphene increases the electroactive surface area and any pretreatment on graphene surface such as graphene doping or oxidation introduce defects on graphene structure resulting in changed electrochemical performance [33]. The repeatability and reproducibility of the sensor are assessed via repetitive measurements performed at the same electrode or different electrodes at the same conditions. For the repeatability analysis, we have measured the signal of 6 μM NIC at the same SPCE for five times and compared the peak current values recorded at each cycle to calculate the relative standard deviation (RSD) between successive measurements. The sensor showed a great repeatability with the RSD < ± 5% without significant signal loss. Reproducibility test, on the other hand, was done via measuring the peak current of 6 μM NIC at 5 different sensors that showed similar response with an RSD < ± 10%. In addition to that, as can be seen in Fig. 4B, small error bars (n = 3) indicating very stable and repeatable behavior of the sensor over a tested concentration range. The electrode stability was tested after 1 and 2 weeks storage at room temperature and the current responses were stable with only ± 5% and ± 10% (n = 5) decrease respectively, indicating 2-weeks shelf life. Therefore, these results indicate that this sensor can be conveniently applied for the sensitive and precise determination of NIC under the selected experimental conditions showing a good repeatability and reproducibility.

3.6. Interference studies

The selectivity of the developed sensor was investigated by performing the measurements in the presence of the interfering compounds at the same concentration as NIC (20 μM). As can be seen in Fig. 5, % of signal.

Tested common ions did not interfere with NIC detection. Organic compounds which are present in the human body fluids such as AA and UA were also tested and their effect was negligible compared to their concentration ranges found in human blood 34–80 and 178–416 μM respectively [41]. However there is no information regarding their range in human sweat. Therefore we have chosen blood concentration ranges as a reference for the measurements we did in human sweat. Also, Cotinine has not shown any significant effect on the current response of NIC. These results prove that, the designed sensor is capable of selective quantification of NIC in human sweat.

3.7. NIC quantification in the sweat samples of heavy/light smokers

To the best of our knowledge, the electrochemical detection of NIC

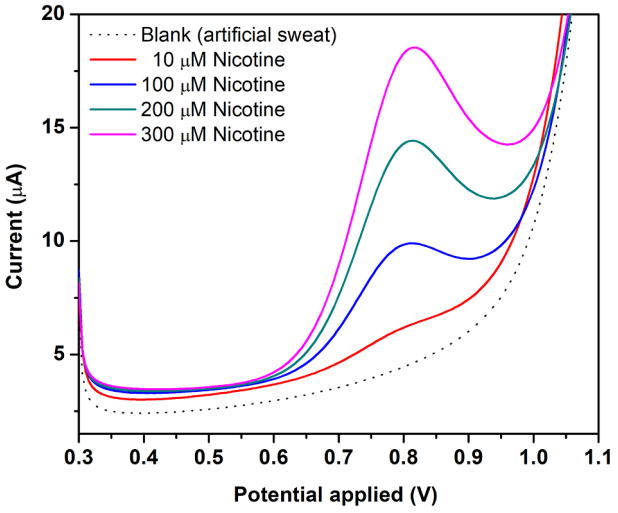

Fig. 6. Differential pulse voltammograms obtained at the unmodified SPCE showing the analysis of the artificial sweat samples in absence (Blank; dashed-line, black color) and in presence of NIC at 10, 100, 200 and 300 μM at pulse amplitude of 140 mV, pulse time 20 ms and scan rate of 50 mV s^{-1}.

Table 2
Quantification of nicotine in artificial sweat solutions based on the proposed method.

Artificial Sweat		
Added (μM)	Found (μM)	Recovery (%)
0.0	0.0	–
10.0	10.1 ± 0.2	101
100.0	90.5 ± 1.4	91
200.0	186.6 ± 1.7	93
300.0	276.0 ± 2.7	92

Table 3
Quantification of nicotine in the real human sweat extracted from sweat patches using the proposed method.

Heavy Smoker			Light Smoker		
Added (μM)	Found (μM)	Recovery (%)	Added (μM)	Found (μM)	Recovery (%)
–	23.4	–	–	4.4	–
10.0	32.3 ± 0.2	97	10.0	15.3 ± 0.8	106
30.0	54.1 ± 0.3	101	30.0	34.6 ± 0.7	101
80.0	106.3 ± 0.9	103	80.0	83.0 ± 1.1	98

in human sweat has not been reported so far. To challenge our sensor to be the first example in literature, we have used our sensors for detection of NIC first in artificial sweat and then in real human sweat collected with a sweat patch. All samples were analyzed in triplicate, and results are given as mean value of three measurements.

Fig. 6 shows voltammograms recorded for various concentrations of NIC standard solution added to artificial sweat for the final NIC concentrations of 10, 100, 200 and 300 μM. It is evident that the oxidation peak of NIC increases proportionally after each addition with a slight shift to more positive potentials compared to the measurements performed in PBS. The high background signal might be attributed to the ions inside the sweat. However, at the oxidation potential, we did not observe any peaks as an interference of other compounds. The recovery experiments were performed in artificial sweat by adding known amounts of NIC inside the solution, recording the signal and calculating the concentration values from calibration curve equation (Eq.3). As can be seen in Table 2, the recovery rates were in the range of 91–101% showing a negligible impact of the complex artificial sweat solution. Having proved the capability of the detection of NIC in more complex media, we tested the sensor in real human sweat. To this aim, first, we collected sweat from heavy and light smokers during their physical activity via sweat patches that have contributed to the development of analytical methods for the analysis of drugs in sweat. The results are shown in Table 2 and 3. The amount of NIC absorbed on the patch of the heavy smoker was higher than on the patch of the light smoker, corresponding to absolute amounts of NIC/patch of 0.047 μmol (7500 ng/patch) and 0.009 μmol (1400 ng/patch) respectively and consistent with literature findings where 29 subjects of passive and light smokers were tested and the nicotine levels were reported to be 150–2498 ng/patch [7]. Spiking of extracts with different nicotine concentrations gave satisfactory recoveries (97 – 106%) showing a promising feasibility of sweat patches for sweat collection for the determination of nicotine in sweat samples.

4. Conclusions

Sweat analysis is a hot topic for the world of “internet-of-things” that aims to connect us with smart sensors/biosensors to monitor physiological parameters. To that aim, various designs have come into being for detection of biomarkers such as glucose, lactate and some ions. On the other hand, there are many other compounds/biomarkers such as Nicotine, if detected could greatly impact the personalized health monitoring. Therefore, in this work, we reported for the first time a very simple electrochemical sensor based on commercially available SPCE for detection of Nicotine from smokers’ sweat collected by commercial sweat collecting patches worn by volunteers who were heavy and light smokers during their physical activities. The sensor represented a very low LOD without any complicated nanomaterial functionalization and is capable of detecting the Nicotine at the physiological levels. In addition to the high sensitivity, the sensor also provides a very good selectivity over multiple interference compounds and hence demonstrate a promising performance for real life scenarios to monitor NIC levels required for smoking cessation or nicotine replacement therapy.

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