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Abstract

Illicit drug consumption is a worldwide worrying phenomenon that troubles modern society. For this reason, law enforcement agencies (LEAs) are placing tremendous efforts into tackling the spreading of such substances among our community. New sensing technologies can facilitate the LEAs duties by providing portable and affordable analytical devices. Herein, we present for the first time a sensitive and low-cost electrochemical method, i.e. square-wave adsorptive stripping voltammetry on carbon screen-printed electrodes (SPE), for the detection of five illicit drugs (i.e. cocaine, heroin, 3,4-methylenedioxymethamphetamine, 4-chloro-alpha-pyrrolidinovalerophenone, and ketamine) in oral fluid by the aid of a surfactant. Particularly, the surfactant is adsorbed at the carbon electrode's surface and yields the adsorption of illicit drug molecules, allowing for an enhanced electrochemical signal in comparison to surfactant-free media. First, the surfactant-mediated behavior is deeply explored at the SPE by cyclic voltammetry, electrochemical impedance spectroscopy, and Fourier-transform infrared spectroscopy. Subsequently, the electrochemical behavior of the five illicit drugs is studied and optimized to render optimal analytical performance. Accordingly, the analytical system exhibited a wide linear concentration range from 1 to 30 µM with sub-micromolar limits of detection and high sensitivity. This performance is similar to other reported electrochemical sensors, but with the advantage of using an unmodified SPE, thus avoiding costly and complex functionalization of the SPE. Finally, the methodology was evaluated in diluted oral fluid samples spiked with illicit drugs. Overall, this work describes a simple, rapid,

portable, and sensitive method for the detection of illicit drugs aiming to provide oral fluid testing opportunities to LEAs.

Keywords

Square-wave adsorptive stripping voltammetry, surfactant, oral fluid testing, screen-printed electrodes, illicit drugs, forensic analysis

1. Introduction

Drugs of abuse is a worldwide worrying issue that hinders the wellbeing of society [1]. In Europe, illicit drugs use was acquainted for 4.3 million people for cocaine, 2.7 million for MDMA, and 1.3 million for heroin in 2019 [2]. Despite the rapid spread of the coronavirus in 2020 and the enforcement of restrictive public health measures, the distribution and consumption of drugs of abuse have not decreased [3]. At first, local drug markets appeared to be affected by social distancing measures, but rapidly, the drug distributors adapted their retail markets to the new measures [2]. Besides, it was observed an increased interest in online sales via darknet markets [3]. Unfortunately, illicit drugs have found their way to consistently being among society, even in times of pandemic.

Law enforcement agencies (LEAs) aim to tackle drug abuse among society via two open frontlines which are the interception of large drug shipments and the identification of the illicit drugs' consumption among users. First, the confiscation of cargo is essential to combat drug trafficking and avoid illicit drugs to reach the market. Therefore, the rapid analysis of potential seizures in border settings is paramount to hinder the spreading of illicit drugs among society [2]. Second, the detection and prevention of the consumption of illicit drugs in drug users are crucial during daily situations: (i) at the workplace [4]; (ii) during driving under the influence of drugs (DUID) [5]; (iii) during therapeutic treatments when the drug is prescribed [6], thus avoiding the misuse of the drug; and (iv) at forensic drug analysis during criminal investigation [7]. In general, the problem is not only ascribed to an unhealthy use of the drug by the consumer, but also to the dangerous situation that is exposed when the drug user is consuming.

Oral fluid is the biofluid selected to perform on-site drug screening as it is easy to collect and handle through non-invasive methods [8]. Besides, pharmacokinetic

parameters are widely correlated with blood drug levels, showing a sharp increase of the drug levels in oral fluid after its consumption [9,10]. In that sense, therapeutic drug monitoring through oral fluid has been proposed for antiepileptic drugs [11] and other therapeutic molecules in infants and neonates [12]. Overall, oral fluid has demonstrated great potential to be used as a biofluid for drug determination.

The screening approach to determine illicit drugs in oral fluid is based on lateral flow assays (LFA) technology [13] (e.g. Dräger DrugTest® 5000 employed by the police officers during roadside tests [14]). LFA offers several advantages such as easy mass fabrication and multiplexing capability [15]. In contrast, LFA usually offers qualitative results, and the use of antibodies as the selective element increases the cost per device [16]. In contrast, electrochemical sensors based on screen-printed electrodes (SPEs) have been proposed for the detection of illicit drugs in oral fluid as they exhibit fast response, miniaturization, affordability, and the possibility of quantification of the analyte [17–19]. Recently, wearable electrochemical sensors have been designed to monitor therapeutic and illicit drugs aiming for seamless integration of the sensors into the body [20]. For example, a ring-based sensor was reported for simultaneous detection of Δ9-tetrahydrocannabinol and alcohol in saliva through electrochemical sensors [21]. Interestingly, a lubricin coating on a SPEs enabled the quantification of clonazepam while avoiding fouling issues [22]. Reaching low limits of detection (LOD) is essential when dealing with oral fluid as they need to achieve the suitable cut-off levels provided by LEAs (in the ng mL^{-1} range) [23]. For this reason, the modification of SPE with molecularly imprinted polymers [24] or the development of affinity-based biosensors [25] have been reported for low detection ranges. Despite these promising results, the challenge is to provide affordable electrochemical sensors that can actually compete with current LFA. Therefore, SPEs with simple modifications that can provide outstanding analytical performance is one of the goals to bring electrochemical sensors into the oral fluid testing.

The presence of surfactants such as sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB) in the electrochemical method was proposed earlier as a rapid and low-cost approach to enhance the analytical performance of electrochemical sensors [26]. Later, surfactants were also used to improve the analytical parameters in the detection of pharmaceutical drugs with pyrolytic graphite electrodes [27], in combination with ionic liquid in carbon paste

electrodes [28], in mixture with nanomaterials [29], and also using SPE [30]. Concerning the use of SDS for the detection of narcotics, morphine [31] nalbuphine and tramadol [32], and 1-(3-chlorophenyl)piperazine [33] have been reported. Still, the enhanced electrochemical detection of popular illicit drugs (e.g. cocaine, heroin, 3,4-methylenedioxymethamphetamine–MDMA) employing SPEs has not been described. Therefore, the combination of SPEs and surfactant-mediated analysis has proven to be a potential method to reach LOD suitable for oral fluid testing.

Herein, we report for the first time the electrochemical analysis of cocaine, heroin, MDMA, 4-chloro-alpha-pyrrolidinovalerophenone (Cl-PVP), and ketamine (**Fig. S1**) with sodium dodecyl sulfate (SDS)-mediated enhancement in oral fluid at unmodified graphite SPEs. Each illicit drug exhibits an electrochemical signal in aqueous media which is significantly enhanced in presence of an optimized concentration of SDS. It is suggested that an accumulation of the protonated drug molecules that electrostatically interacts with negatively charged structures of the SDS enhances the electrochemical signal allowing sub-micromolar LODs at unmodified SPEs. First, the SDS-mediated behavior is deeply explored at the SPE by electrochemical methods proving an adsorption-controlled process at the surface of the SPE. Besides, the adsorption effect of the SDS on the SPE surface is assessed by Fourier-transform infrared spectroscopy (FTIR). Subsequently, the analytical performance of the five illicit drugs is evaluated under optimal conditions (i.e. pH, time of adsorption) using square-wave adsorptive stripping voltammetry (SWAdSV). Finally, the methodology was interrogated under oral fluid samples spiked with the corresponding illicit drugs. Overall, this work describes a rapid, portable, and sensitive method for the detection of illicit drugs aiming to provide oral fluid testing opportunities to LEAs.

2. Materials and methods

2.1. Materials

Standards of cocaine·HCl, heroin·HCl, were purchased from Chiron AS, Norway. Standards of 4-chloro-alpha-pyrrolidinovalerophenone·HCl (Cl-PVP), and 3,4-methylenedioxymethamphetamine·HCl (MDMA), and ketamine·HCl were purchased from Lipomed, Switzerland. Analytical grade salts of potassium chloride, potassium phosphate, sodium borate, sodium acetate, potassium ferricyanide, and potassium

hydroxide were purchased from Sigma-Aldrich (Overijse, Belgium). Sodium dodecyl sulfate (SDS) was purchased from Sigma-Aldrich (Overijse, Belgium).

All solutions were prepared in 18.2 MΩ cm^{-1} doubly deionized water (Milli-Q water systems, Merck Millipore, Germany). The pH was measured using a pH-meter (914 pH/Conductometer, 2.914.0020, Metrohm, Switzerland).

2.2. Methods

Electrochemical profiles were recorded using a MultiPalmSens4 (PalmSens, The Netherlands) with PSTrace/MultiTrace. Disposable ItalSens screen-printed electrodes (SPE) (PalmSens, the Netherlands), containing a graphite working electrode (Ø = 3 mm), a carbon counter electrode, and a (pseudo) silver reference electrode were used for all measurements. Cyclic voltammetry (CV) parameters used: scan rate of 0.1 V s^{-1} with a step potential of 0.010 V s^{-1}. Electrochemical impedance spectroscopy (EIS) parameters that were used: E_{dc}=0.2 V, E_{ac}=5 mV, frequencies= 0.1 Hz-100 KHz when using the ferricyanide probe at 2 mM; and E_{dc}=0.9 V, E_{ac}=5 mV, frequencies= 0.1 Hz-100 KHz when interrogating cocaine sample at 50 µM. EIS signals were analyzed based on Randles equivalent circuit R_s-([R_{ct}W]C_{dl}), where R_s is the solution-phase resistance, R_{ct} is the charge transfer resistance, C_{dl} is the double-layer capacitance and W is the Warburg impedance that is related to the mass transfer effect. The parameters were obtained by PSTrace software fitting the points to a semicircle. The square-wave voltammetry (SWV) parameters that were used: potential range of 0.0 to 1.2 V, frequency 10 Hz, 25 mV amplitude, and 5 mV step potential. SWAdSV used the same parameters for the SWV but leading a time of adsorption upon launching the SWV. Voltammograms are background corrected using the “moving average correction” (peak width = 1) tool in the PSTrace software. Electrochemical tests were performed in 20 mM Britton Robinson buffer (BR) solutions with 100 mM KCl at a suitable pH by applying 50 µL of the solution onto the SPE. Phosphate buffer saline (PBS) at pH 7 with ferricyanide as a redox probe was used for the CV and EIS analysis. The SDS-mediated buffer solutions were prepared from a fresh stock of 10 mg mL^{-1} SDS.

An Attenuated Total Reflectance (ATR) FTIR spectrometer (Bruker Alpha 2, the UK with a diamond crystal) was used to evaluate the SDS adsorption on the surface of the SPE. An FTIR spectrum from the SDS powder was first acquired for comparison

with the water-based samples. Thereafter, the spectrum acquired by a sample consisting of water drop cast on the SPE was used as the background data for the following FTIR experiments with SDS in solution. Subsequently, 0.2, 2, and 10 mg mL^{-1} SDS solutions in water were employed at the SPE. For each spectrum, 128 scans were accumulated at 4 cm^{-1} spectral resolution, the wavenumber range was between 4000 to 475 cm^{-1}. The spectra have been baseline-corrected, no additional treatments have been applied. The corrections of the spectra have been executed using the OPUS 8.2 software.

2.3. Illicit drugs detection in oral fluid

Oral fluid samples (from fasting subjects or at least 2 to 3 hours after consuming food or taking any medication) were collected immediately before analysis by spitting into a 3 mL tube. A centrifugation step (i.e. 10.000 rpm for 1 min) was evaluated to remove potential debris from the oral fluid during the optimization process. Different aliquots of the oral fluid were taken in 1.5 mL tubes and were spiked with one of the illicit drugs. The process was repeated for each one of the illicit drugs at the test. For cocaine, spiked samples from 10, 25, 50, 100, 150, and 200 µM were prepared; for heroin, 50, 100, 150, 200, 300, and 400 µM were prepared; for MDMA, spiked samples from 25, 50, 100, 150 and 200 µM were prepared; and finally, for ketamine, spiked samples from 50, 100, 200, 300 and 400 µM were prepared. Next, each spiked saliva sample was diluted 10-fold in the corresponding buffer-containing SDS, and electrochemically interrogated with a SPE.

3. Results and discussion

3.1. Exploration of the SDS effect at the SPE.

The influence of the presence of SDS on the electrochemical detection at the SPE was evaluated by CV and EIS (**Fig. 1**). It is expected that the SDS molecules are adsorbed on the surface of the SPE due to the hydrophobicity of the graphite ink which interacts with the non-polar chain of the surfactant, thus altering the electrochemical signal obtained [34]. Besides, the high ionic strength of the solution suggested a more compact SDS adsorbate structure on the surface. First, voltammograms in potassium ferricyanide solution were recorded to assess the redox behavior of the probe at the SDS/SPE surface. It is proposed that the adsorption of the 12-carbon tail onto the surface of the SPE exposes the sulfate group of the anionic surfactant, creating a

negatively charged layer [34]. **Fig. 1a** shows the decrease in the peak current (I_p) from 36.6 µA to 27.4 µA at the oxidation peak due to the presence of SDS. Simultaneously, a separation of the redox peak-to-peak potential from 170.2 mV to 420 mV took place. **Table S1** displays the corresponding parameters of I_p and peak-to-peak potential from the CVs with and without SDS in PBS pH 7. The results suggest that the negatively charged layer adsorbed at the SPE hindered the diffusion of ferricyanide (negatively charged) onto its surface. The electron transfer properties were also studied by EIS. **Fig. 1b** exhibits the Nyquist plot in the ferricyanide solution showing an increment in the charge transfer resistance from 5.1 kΩ (SPE) to 11.3 kΩ (SDS/SPE), thus exhibiting a decrease in the kinetic rate (**Table S2**). Overall, the CV and EIS analysis showed evidence of adsorption of the SDS molecules at the surface of the SPE.

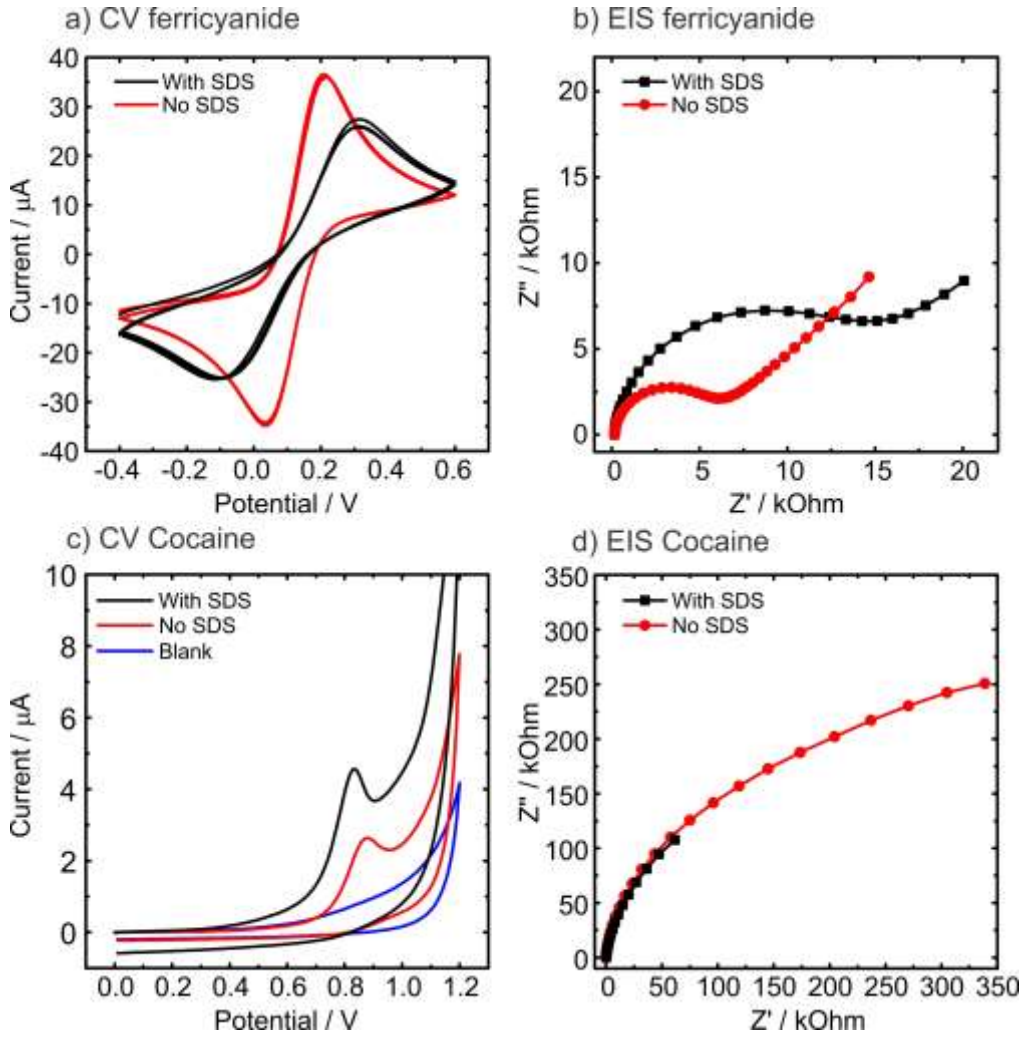

Fig. 1. *Electrochemical exploration of SDS-mediated measurements at the SPE: a) CV curves and b) Nyquist plot of solutions with and without SDS in 2 mM potassium ferricyanide PBS pH 7 with 0.1 mg mL^{-1} SDS. EIS was performed under the following*

parameters: E_{dc}=0.2 V, E_{ac}=5 mV, frequencies= 0.1 Hz-100 KHz.; c) CV curves and d) Nyquist plot of solutions with and without SDS in 50 µM cocaine BR pH 9 with 0.1 mg mL^{-1} SDS. EIS was performed under the following parameters: E_{dc}=0.9 V, E_{ac}=5 mV, frequencies= 0.1 Hz-100 KHz. CV=cyclic voltammetry; EIS=electrochemical impedance spectroscopy.

As the target analytes of this work are illicit drugs, cocaine was selected as a model to explore the SDS-mediated electrochemical approach. **Fig. 1c** displays CVs of 50 µM cocaine in BR pH 9 with and without SDS in solution exhibiting a non-reversible process. In contrast to previous results with ferricyanide analysis, the peak potential of cocaine oxidation shifts toward less positive potentials when using SDS, and the peak current increases from 2.6 µA (SPE) to 4.6 µA (SDS/SPE) (**Table S1**). Besides, the EIS analysis presented a dramatic decrease in the charge transfer resistance from $1.5*10^5$ Ω (SPE) to 0.2 Ω (SDS/SPE) (**Table S2**). Therefore, the electrochemical process of cocaine oxidation with SDS in solution clearly enhances the electron transfer rate at the SPE surface (**Fig. 1d**). According to the literature [34], the SDS hemimicelles oriented along the graphitic surface of SPEs might offer a beneficial environment for the adsorption of the lipophilic drugs which can easily accumulate at the electrode's surface, and consequently, facilitate the catalytic effect and enhance the current output. The accumulation of cocaine molecules at the surface of the SPE by the SDS exhibits faster charge transfer kinetics at the interface, thus enhancing the oxidation reaction.

Apart from SDS, other commonly used surfactants were studied under electrochemical interrogation at different pH. SWV was subsequently used as it provides rapid and sensitive results. Tween 20 was selected as a non-ionic surfactant and cetyl trimethyl ammonium bromide (CTAB) was used as a cationic surfactant. **Fig. S2a** and **Fig. S2b** display the electrochemical profiles of 50 µM cocaine with 0.1 mg mL^{-1} of each surfactant at pH 12 and pH 7, respectively. The electrochemical examination was performed at both pHs to evaluate whether the protonated or deprotonated form of the illicit drug would alter the analytical response depending on the interaction with a non-ionic, cationic, or anionic surfactant. The results showed that SDS enhance the electrochemical signal at pH 7 due to its proposed interaction of the protonated form of cocaine with the negatively charged heads of SDS, while Tween 20 and CTAB did

not produce a noticeably increase in the signal. Overall, SDS was selected for further optimization.

As pH 7 exhibited an enhancement of the electrochemical response, the effect of the SDS concentration in the buffer was evaluated. **Fig. S2c** and **Fig. S2d** show the SWV and the baseline-corrected SWV of 50 µM cocaine pH 7 at different concentrations of SDS (0, 0.01, 0.05, 0.1, 0.2, and 0.5 mg mL^{-1}). Despite the increase in the background current upon increasing the SDS concentration, the I_p also increased until reaching a plateau at 0.2 mg mL^{-1} (**Fig. S2e**). Therefore, 0.2 mg mL^{-1} was chosen as the SDS concentration with optimal signal/noise ratio. For the following experiments, the baseline-corrected SWVs are used to compare the electrochemical profiles.

ATR–FTIR spectroscopy was subsequently employed to investigate the adsorption of SDS at the electrode's surface. First, **Fig. S3a** shows the FTIR spectrum of the SDS in powder form to determine the spectral region of interest and the characteristic bands from the SDS: (i) from 900 to 1300 cm^{-1} which includes the S–O stretching bands, and (ii) from 2800 to 3000 cm^{-1} which indicates the CH stretching bands [35]. **Fig. S3b** displays the FTIR spectra of adsorbed SDS at SPE surface from aqueous solutions at 0.2, 2, and 10 mg mL^{-1} concentrations, showing a similar profile to SDS adsorbed on other surfaces [36]. The same bands can be observed at the SPE upon incubation with SDS aqueous solution in comparison to the standard profile (**Fig. S3a**) with only small shifts of the bands which can be attributed to the interactions with the graphite surface. Therefore, the adsorption of SDS in an aqueous solution is demonstrated on the graphite SPE surface. Note that the SDS bands are prominent at high SDS concentration, although it is also expected adsorption at lower SDS amounts.

3.2. Electrochemical behavior of illicit drugs using SDS-mediated solution.

SWV was employed to investigate the electrochemical behavior of the illicit drugs (i.e. cocaine, heroin, MDMA, Cl-PVP, and ketamine, **Fig. S1**) on a SPE in the presence of SDS. When SDS (0.1 mg mL^{-1}) was present in the BR solution of 50 µM of the illicit drug at pH 7, the SWV exhibited a clear enhancement in the peak current and a slight shift in the peak potential toward less positive potentials (**Fig. 2**). Despite an increment in the background current shown in the raw SWV (**Fig. S4**), the SDS clearly improves the electrochemical oxidation of the illicit drugs at the SPE. Particularly, cocaine showed a 5.2-fold, heroin a 2.5-fold, MDMA a 2.1-fold, Cl-PVP a 3.5-fold, and

ketamine a 4.9-fold enhancement in comparison to the electrochemical analysis in BR pH 7 without SDS in the solution (**Table S3**). The electrochemical profile of cocaine shows a redox peak suggesting the oxidation of its tertiary amine moiety at ca. 0.8 V (**Fig. 2a**) [37]. Similarly, the heroin profile exhibits a redox peak at ca. 0.9 V corresponding to the oxidation of the tertiary amine in its structure (**Fig. 2b**) [38]. The MDMA profile displays a single oxidation peak at ca. 0.95 V suggesting the oxidation of its methylenedioxy group at pH 7 [39] (**Fig. 2c**). Interestingly, the electrochemical profile of Cl-PVP shows two oxidation peaks corresponding to the oxidation of its cyclic amine at ca. 0.70 V, as well as with subsequent oxidation of one of its products at ca. 0.87 V [40] (**Fig. 2d**). Lastly, the ketamine profile exhibits a redox peak at ca. 0.95 V corresponding to the oxidation of its secondary amine [41] (**Fig. 2e**). Importantly, the blank experiments with only SDS in solution did not show any electrochemical contribution from SDS (**Fig. 2**), thus indicating that the use of SDS is a viable approach to enhance the electroanalytical parameters for illicit drug detection employing unmodified graphite SPEs.

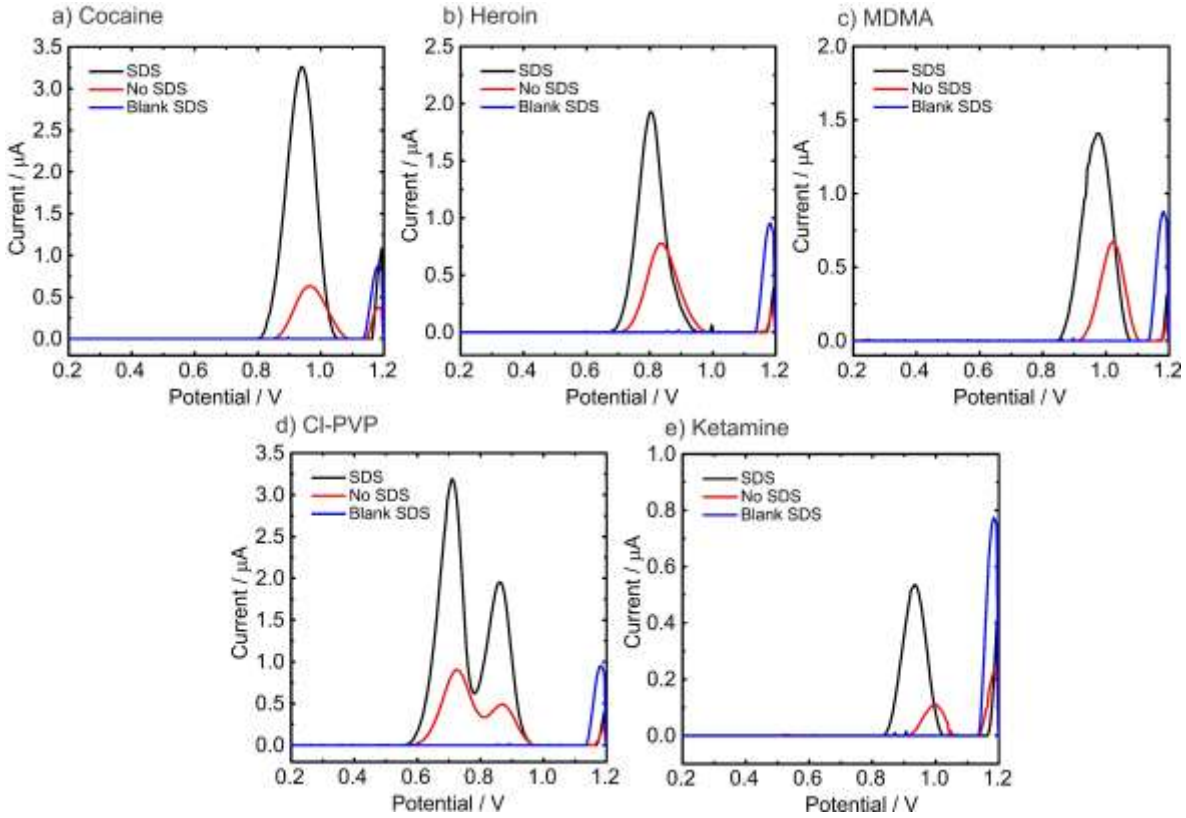

Fig. 2. *Enhancement of the electrochemical signal using SDS-mediated solution (0.1 mg mL^{-1}) by SWV in BR pH 7 at 50 µM of each illicit drug: a) Cocaine, b) heroin, c) MDMA, d) Cl-PVP, and e) ketamine. SWVs were baseline-corrected.*

To find the best condition for the most sensitive SWV analysis and to understand the potential pH dependence of the method, a pH screening of 50 µM of each illicit drug was performed (**Fig. S5**). All the illicit drugs exhibit a pH dependency on the peak potential (E_p), shifting towards lower potentials when the SWV analysis is performed in more alkaline pH's, and consequently, indicating protons participation in the electrochemical oxidation process. **Fig. S5a** and **Fig. S5b** display the SWVs, and the I_p and E_p dependence on pH from the electrochemical oxidation of cocaine at SDS/SPE, respectively. A linear relationship was observed between pH 6 to pH 9 from E_p following E_p (V) =−0.073 pH + 1.46, reaching a plateau from pH 9 to pH 12 due to the deprotonated tertiary amine (pK_a=8.61). Between pH 6 to pH 9, cocaine oxidation involves the transfer of an equal amount of protons and electrons ($2e^-/2H^+$). Concerning heroin, **Fig. S5c** and **Fig. S5d** show the SWVs, and the I_p and E_p dependence on the pH, respectively. Similarly, the pH dependency on the E_p follows E_p (V) =−0.074 pH + 1.33 from pH 4 to pH 8 reaching a plateau from pH 9 to pH 12 (pK_a=7.95), suggesting the involvement of an equal amount of protons and electrons in the oxidation process ($2e^-/2H^+$). **Fig. S5e** and **Fig. S5f** exhibit the SWVs, and I_p and E_p dependence on the pH for MDMA, respectively. In this case, the E_p dependency on the solution pH shows two ranges: (i) from pH 4 to pH 7 following E_p (V) =−0.027 pH + 1.17, showing half of Nernstian slope (i.e. 0.059 V pH^{-1} at 298 K), which indicates the transfer of a proton and two electrons in the electrochemical oxidation process; and (ii) from pH 8 to pH 11 following E_p (V) =−0.075 pH + 1.47, suggesting the transfer of an equal amount of protons and electrons ($2e^-/2H^+$). It is suggested that the first process is in relation to the oxidation of the methylenedioxy group, and the second relationship is related to the oxidation of the secondary amine (pK_a=9.9). **Fig. S5g** and **Fig. S5h** display the SWVs, and the I_p and E_p dependence on the pH for Cl-PVP, respectively. From pH 6 to pH 9, the E_p of Cl-PVP (at ca. 0.70 V) exhibits a linear relationship of E_p (V) =−0.069 pH + 1.21, suggesting the involvement of equal transfer of proton and electrons ($2e^-/2H^+$) matching a reported oxidation pathway [40]. Finally, **Fig. S5i** and **Fig. S5j** show the SWVs, and the I_p and E_p values from the pH screening of ketamine, respectively. The linear relationship of E_p (V) =−0.065 pH + 1.40 from pH 6 to pH 8 shows the transfer of an equal amount of protons and electrons in the oxidation process ($2e^-/2H^+$) as previously reported [41]. A small shift of the E_p was obtained from pH 9 to pH 12, suggesting no proton transfer, as the secondary amine is deprotonated (pK_a=7.5).

According to the highest value for I_p obtained during the pH screening, pH 9, pH 6, pH 5, pH 9, and pH 8 were selected as the optimal pH for cocaine, heroin, MDMA, Cl-PVP, and ketamine, respectively. The optimal pH values are below or coincide with the pK_a values of the illicit drugs, suggesting that the protonated form of the drug improves the sensitivity of the detection method. This fact could be explained due to the interaction of protonated drugs with the negatively charged moieties of SDS which, in turn, might facilitate the accumulation of the illicit drug on the surface, enhancing the oxidation signal. In general, the polarity of the illicit drug at each pH (protonated state) will also determine the interaction between SDS and the molecule which will facilitate the oxidation process at the SPE surface.

The study of the phenomenon occurring at the SPE surface during the electrochemical reaction is essential to understand the sensing concept before testing the analytical performance of the approach. Therefore, a scan-rate study with CV from 0.025 to 0.6 V s^{-1} was executed for each illicit drug at 50 µM with SDS under the optimal pH (**Fig. 3a** for cocaine, **Fig. 3b** for heroin, **Fig. 3c** for MDMA, **Fig. 3d** for Cl-PVP, and **Fig. 3e** for ketamine). For the proposed illicit drugs, the I_p increases upon higher scan rates, as well as there is a shift toward positive potentials. In the voltammograms, the only presence of oxidation peaks indicates an irreversible process for all illicit drugs. According to the results in the CV exploration, a linear relationship was obtained between the I_p and the scan rate, suggesting that the electrochemical reaction is governed by an adsorption-controlled process for all the drugs (**Fig. 3f** for cocaine, I_p (µA) = 86.5 v (V s^{-1}) +1.8 µA, R^2 =0.99; **Fig. 3g** for heroin, I_p (µA) = 77.9 v (V s^{-1}) +3.2 µA, R^2 =0.99; **Fig. 3h** for MDMA, I_p (µA) = 94.5 v (V s^{-1}) +3.7 µA, R^2 =0.99; **Fig. 3i** for Cl-PVP, I_p (µA) = 50.9 v (V s^{-1}) +1.2 µA, R^2 =0.99; and **Fig. 3j** for ketamine, I_p (µA) =66.2 v (V s^{-1}) +1.9 µA, R^2 =0.99). Besides, the logarithm of I_p and the logarithm of the scan rate were plotted. A slope higher than the theoretical value of the charge transfer coefficient (α=0.50, corresponding to diffusion-controlled process) is expected. In this way, **Fig. S6a** shows a slope of 0.83 for cocaine, **Fig. S6b** a slope of 0.79 for heroin, **Fig. S6c** a slope of 0.77 for MDMA, **Fig. S6d** a slope of 0.87 for Cl-PVP, and **Fig. S6e** a slope of 0.82 for ketamine, thus clearly indicating an adsorption process. In order to confirm these findings, the I_p was plotted against the square root of the scan rate (**Fig. S7**). In this case, a linear relationship should be presented for a diffusion-controlled process. As the SDS system is an adsorption process, the

relationship indicated a non-linear correlation, thus confirming the previous calculations. Overall, the SDS addition in the buffer solution alters the electrochemical mechanism from diffusion-controlled process (e.g. cocaine [37]) to adsorption-controlled process at SDS/SPE, thus opening new possibilities for the optimization of the analytical performance.

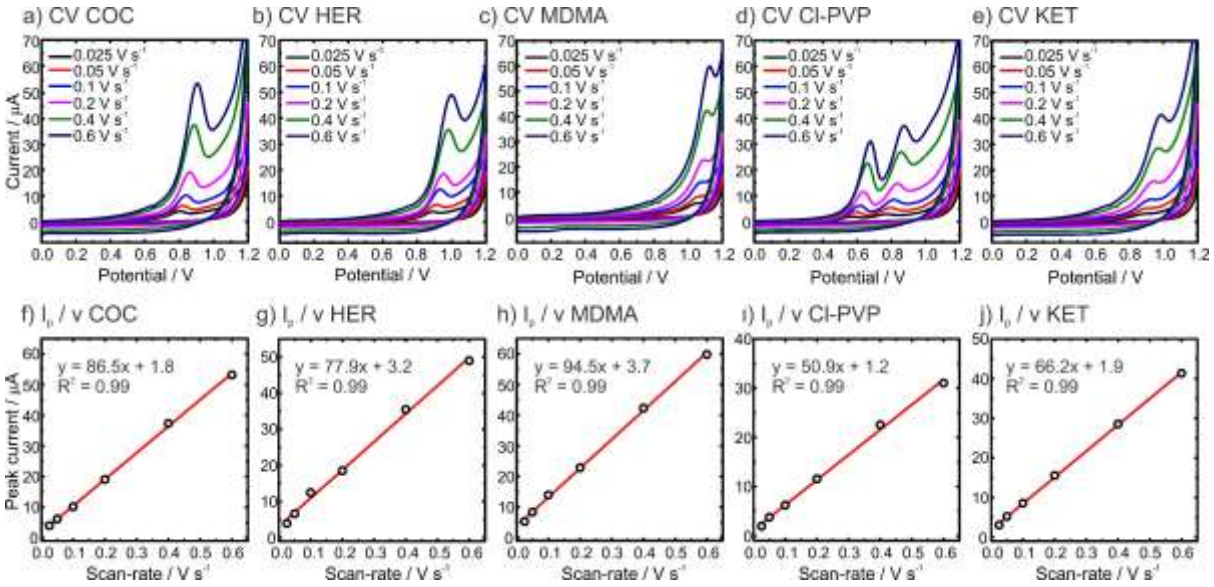

Fig. 3. *Investigation of the electrochemical phenomena at the SDS/SPE system: CV curves of 50 µM illicit drug at different scan-rates (0.025, 0.05, 0.1, 0.2, 0.4, and 0.6 V s^{-1}) of a) cocaine, b) heroin, c) MDMA, d) Cl-PVP, and e) ketamine at pH 9, pH 6, pH 5, pH 9, and pH 8, respectively, with SDS. The corresponding relationship of the peak current with the scan rate for f) cocaine, g) heroin, h) MDMA, i) Cl-PVP, and j) ketamine obtained from the voltammograms. ~~The corresponding relationship of the logarithm of the peak current with the logarithm of the scan rate for k) cocaine, l) heroin, m) MDMA, n) Cl-PVP, and o) ketamine.~~COC=cocaine; HER=heroine; I_p=peak potential; KET=ketamine; v=scan-rate.*

3.3. Analytical performance of the detection of the illicit drug at SDS/SPE.

The nature of the system indicated an adsorption-controlled process. Hence, the influence of the adsorption time in the SWV output was first evaluated before the complete characterization of the analytical parameters (**Fig. S8**). SWVs of 50 µM of the illicit drug at different adsorption times (i.e. 0, 1, 2.5, 5, 10 and 20 min) with 0.2 mg mL^{-1} SDS were tested (**Fig. S8a** for cocaine, **Fig. S8c** for heroin, **Fig. S8e** for MDMA, **Fig. S8g** for Cl-PVP, and **Fig. S8i** for ketamine). Accordingly, the I_p of each SWV

during the time analysis of each drug were displayed (**Fig. S8b** for cocaine, **Fig. S8d** for heroin, **Fig. S8f** for MDMA, **Fig. S8h** for Cl-PVP, and **Fig. S8j** for ketamine). The electrochemical signals increased the I_p upon incubation with the illicit drug through time, reaching a plateau between 10 to 20 min. The exception was Cl-PVP which I_p continuing to increase after 10 min. Further adsorption time was not assessed, as the aim of the sensing concept is to be used in on-site applications in which a reduced time of operation is essential. As a result, the optimal adsorption time was set at 10 min as a balance between sensitivity and time of operation.

The analytical parameters of the sensing concept for the detection of illicit drugs were evaluated by SWAdSV (**Fig. 4**). First, the SDS/SPE was interrogated with increasing concentrations of the illicit drugs (i.e. from 0.5 to 30 µM) at its optimal pH, adsorption time (i.e. 10 min), and SDS concentration (i.e. 0.2 mg mL^{-1}). The upper level of 30 µM was selected as a high concentration encountered in oral fluid during drug consumption, although it might have huge variation depending on the route of administration and time after consumption [9,42]. **Fig. 4a-e** exhibit the dynamic SWV curves for cocaine, heroin, MDMA, Cl-PVP, and ketamine upon increasing concentrations. The I_p of each SWV was displayed according to each concentration to evaluate the linearity of the sensor. **Fig. 4f-j** show the corresponding calibration curve: **Fig. 4f** for cocaine presenting a linear range (LR) from 1 to 30 µM at E_p= 0.83 V, with a sensitivity of 0.40 µA $µM^{-1}$, and a LOD of 0.7 µM –determined by the formula: LOD = 3.3(Sy/S)–; **Fig. 4g** for heroin exhibiting a LR from 2.5 to 30 µM at E_p= 0.92 V, with a sensitivity of 0.24 µA $µM^{-1}$, and a LOD of 1.8 µM; **Fig. 4h** for MDMA showing a LR from 1 to 30 µM at E_p= 1.00 V, with a sensitivity of 0.31 µA $µM^{-1}$, and a LOD of 0.9 µM; **Fig. 4i** for Cl-PVP presenting a LR from 2.5 to 30 µM at E_p= 0.59 V, with a sensitivity of 0.25 µA $µM^{-1}$, and a LOD of 1.6 µM; and **Fig. 4j** for ketamine exhibiting a LR from 2.5 to 30 µM at E_p= 0.87 V, with a sensitivity of 0.10 µA $µM^{-1}$, and a LOD of 1.1 µM.

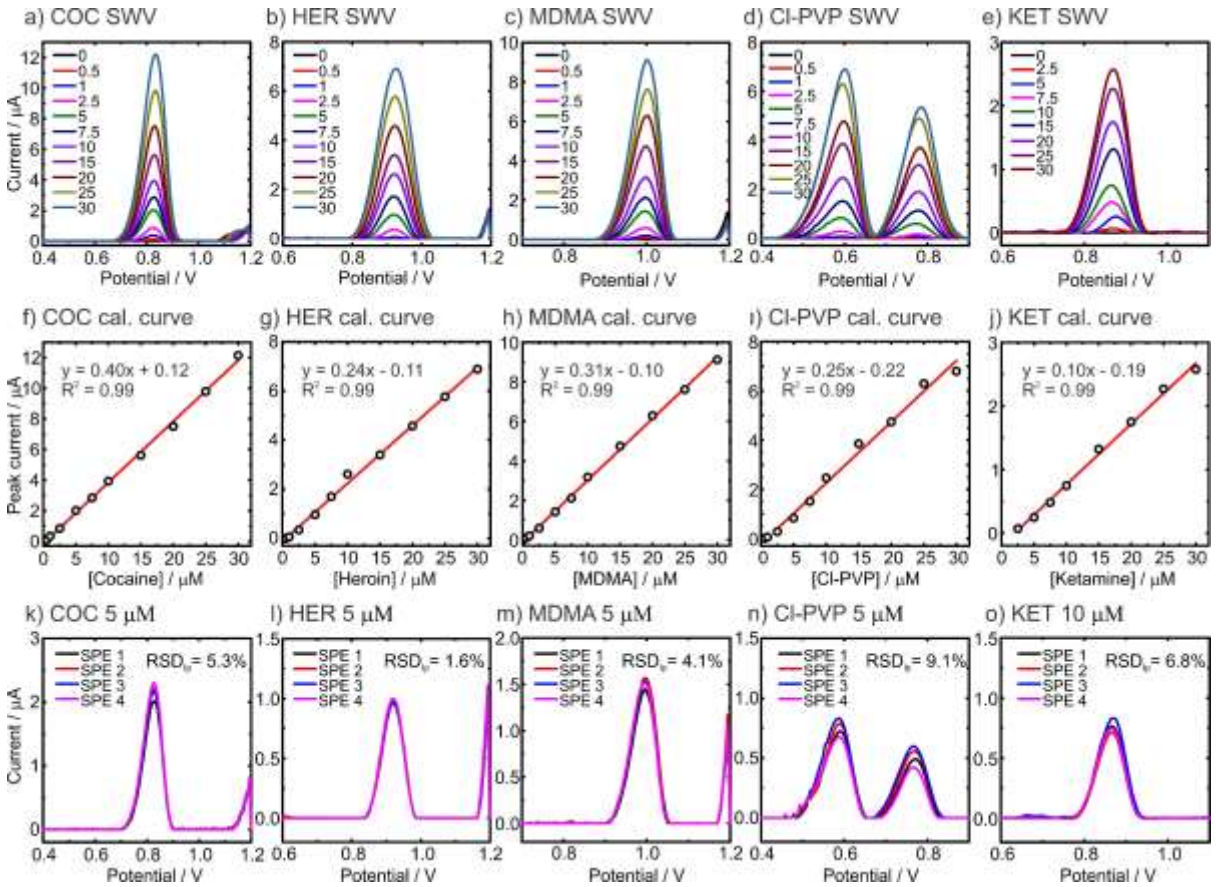

Fig. 4. *Analytical performance of the sensing concept at SDS/SPE by SWAdSV: dynamic curves of increasing concentrations from 0.5 to 30 µM for a) cocaine; b) heroin, c) MDMA, d) Cl-PVP, and e) ketamine. Corresponding calibration curves for f) cocaine; g) heroin, h) MDMA, i) Cl-PVP, and j) ketamine. Intra-day reproducibility SWVs for k) cocaine; l) heroin, m) MDMA, n) Cl-PVP, and o) ketamine; N=4. SDS concentration at 0.2 mg mL^{-1} in all the experiments. 10 min time of adsorption. The tests were executed using BR buffer at pH 9, pH 6, pH 5, pH 9, and pH 8 for cocaine, heroin, MDMA, Cl-PVP, and ketamine, respectively. COC=cocaine; HER=heroine; KET=ketamine.*

The intra-day reproducibility was also assessed for the detection of the illicit drugs with the SDS/SPE at optimal conditions. **Fig. 4k-o** displays the SWVs reproducibility test for cocaine (RSD=5.3%, at 5 µM, N=4); heroin (RSD=1.6%, at 5 µM, N=4); MDMA (RSD=1.5%, at 5 µM, N=4), Cl-PVP (RSD=9.1%, at 5 µM, N=4), and ketamine (RSD=6.8%, at 10 µM, N=4). Besides, the inter-day reproducibility was also evaluated for each illicit drug during 6 days at 5 µM: cocaine (RSD=10.1%); heroin (RSD=7.9%); MDMA (RSD=14.0%), and ketamine (RSD=6.4%); and at 10 µM: cocaine (RSD=8.7%); heroin (RSD=8.7%); MDMA (RSD=9.5%), and ketamine (RSD=11.0%).

In the case of Cl-PVP, the inter-day reproducibility exhibited high values demonstrating a potential drawback for the quantification of the synthetic cathinone in the field. Apart from Cl-PVP, the electrochemical concept involving SDS as an enhancer demonstrates to be highly reliable and robust for the detection of illicit drugs through SPEs.

Finally, the analytical performance was compared to other reported electrochemical sensors based on SPE for the detection of illicit drugs in oral fluid (**Table 1**). The electrochemical sensing concept reported in this work shows excellent features accompanied by the affordability and simplicity of the approach. In comparison, this method: (i) enhances the parameters obtained in unmodified electrodes; (ii) exhibits similar features reported in sensors with complex and time-consuming modifications, and importantly, (iii) it offers a simple and affordable approach for the analysis in oral fluid samples.

Table 1. *Screen-printed electrodes for illicit drugs detection in oral fluid.*

Method	Electrode	Illicit drug	LOD / µM	LR / µM	Reproducibility / %	Ref
SWV	MWCNTs/SPE	THC	0.5	1–6	2.7	[21]
CA	mediated-SPE	THC	0.1–0.16	-	-	[43]
DPV	MIPs/MOF/GPH/SPE	Ketamine	$4x10^{-5}$	$4x10^{-5}$–40	3.2	[24]
SWV	LUB/rGO/SPE	Clonazepam	0.02	0.025–2.5	-	[22]
SWV	mediated-SPE	METH	2.7	up to 33.5	-	[44]
CA	Ab-HRP/SPE	MDMA	$5.3x10^{-3}$	0.1–2.1	7	[45]
SWV	Ab-MB/SPE	Cocaine	$4.9x10^{-7}$	up to 3.3	-	[19]
SWV	MIPs/PdNP/GPH/SPE	Cocaine	50	100–500	0.7%	[46]
SWAdSV	anodic preated-SPE	Fentanyl	0.1	0.9–20.5	2.6	[18]
SWV, CV	SPE	MDMA	9.1	9.1–103.4	-	[47]
SWAdSV	SDS-SPE	Cocaine	0.7	1–30	5.3	This work
		Heroin	1.8	2.5–30	1.6	
		MDMA	0.9	1–30	1.5	
		Cl-PVP	1.6	2.5–30	9.1	
		Ketamine	1.1	2.5–30	6.8	

[a]Accuracy; Abbreviations: Ab: antibody; SWAdSV: Square-wave adsorptive stripping voltammetry; CA: chronoamperometry; CNT: carbon nanotubes; CV: cyclic voltammetry; DPV: differential pulse voltammetry; GPH: graphene; HRP: horseradish peroxidase; LR: linear range; LUB: lubricin; MDMA: methylenedioxymethamphetamine; METH: methamphetamine; MIPs: molecularly imprinted polymers; MB: magnetic beads; MOFs: metal-organic framework; MWCNTs: multi-walled carbon nanotubes; PdNPs: palladium nanoparticles; rGO: reduced graphene oxide; SPE: screen-printed electrode; SWV: square-wave voltammetry; THC: tetrahydrocannabinol.

3.4. Application of the sensing concept in the analysis of oral fluid.

Oral fluid is a complex biofluid consisting of ca. 99% of water, inorganic, and organic substances as well as with a high-loading of proteins such as enzymes, mucin, and albumin [48]. The main strengths of oral fluid in comparison to blood samples or other biofluids are: (i) the easiness in the collection process of an adequate amount which can be supervised by LEAs; (ii) saliva contains the free fraction of the drug, which efficiently reflects the drug physiological activity and state of intoxication [8]; (iii) the levels of the drug in saliva are usually correlated with levels in blood/serum [9,10], and importantly, (iv) with impairment symptoms [23]. In contrast, the main weaknesses are: (i) oral fluid samples are subject to bacterial degradation over time; (ii) the oral fluid might contain food, legal drugs, and other debris from the mouth which might interfere in the analytical process; (iii) the physiological status of the person at test (for example, after drug consumption) might influence the availability of the fluid and its physical properties (e.g. high viscosity), hardening the on-site test; and (iv) the quantification of the analyte might be difficult as accurate sampling volume is needed. Moreover, it is essential to consider the challenges in the electrochemical analysis of oral fluid: (i) the consumption of other electrochemically active might interfere in the electrochemical profile of the illicit drugs; and (ii) the presence of high amount of protein might introduce biofouling issues at the surface of the SPE, disturbing the electron transfer process, and consequently, hampering the quantification of the illicit drug. Despite oral fluid being a complicated matrix for the direct electrochemical detection of drugs, many efforts towards the development of (bio)sensors have been put forward [8,49].

In this work, these challenges are addressed by diluting the oral fluid in BR at the optimal pH for each illicit drug. This dilution with the buffer maintains the ionic strength and pH of the solution, as well as decreases the protein loading in the sample which avoids biofouling issues. At first, SWV was used to assess the matrix effect by 2-fold and 10-fold dilutions of the oral fluid in BR buffer (**Fig. S9**). In this case, pH 9 was selected as a model pH because two drugs in this study are optimal in this condition. The 2-fold dilution exhibited high background current and several oxidation peaks which would overlap with the E_p of the illicit drugs. In contrast, the 10-fold dilution showed a similar SWV profile than BR buffer, exhibiting only an oxidation process at 0.5 V which is out of the oxidation potential window of the illicit drugs (i.e. 0.7 – 1.0 V).

This oxidation process at ca. 0.5 V is attributed to the albumin oxidation present in oral fluid (**Fig. S10a**). Moreover, the albumin oxidation is also pH-dependent (**Fig. S10b**), meaning that the peak potential shifts toward negative potential as the pH increases. This behavior (i.e. shift the peak potential according to the pH of the solution) is similar to the electrochemical oxidation of the illicit drugs (**Fig. S5**), thus avoiding any possible peak overlap between the target molecules and albumin. As a result, the 10-fold dilution was selected for further testing. Subsequently, two methods for the sample preparation were evaluated employing oral fluid samples from two subjects: (i) direct 10-fold dilution in the buffer, and (ii) previous centrifugation of the oral fluid sample and subsequent 10-fold dilution. **Fig. S11a** displays the SWV of both sample preparations showing similar behavior. Accordingly, **Fig. S11b** depicts the baseline-corrected SWVs revealing a small oxidation process at 0.9 V for the samples previously treated with a centrifugation step, which is close to the oxidation potential of the illicit drugs, and non-redox processes at such potentials when using directly the oral fluid sample. Therefore, the direct 10-fold dilution of the oral fluid sample after spitting was selected as the optimal method, avoiding the use of a centrifugation step which is unsuitable for on-site testing. Besides, five replicates of diluted oral fluid samples were evaluated by SWV (**Fig. S11c**) and baseline-corrected (**Fig. S11d**) to demonstrate the lack of an oxidation process at the potential window of interest for the detection of the illicit drugs (0.7 – 1.0 V).

Table 2. *Reported levels of illicit drug in oral fluid and plasma.*

Illicit drug	**Oral fluid concent. / ng mL^{-1}**	**Oral fluid concent. / µM**	**Ref.**	**Plasma concent. / ng mL^{-1}**	**Plasma concent. / µM**	**Ref.**
Cocaine	22 – 23592	0.07 – 77.07	[9,50]	54 – 372	0.18 – 1.23	[9,50]
Heroin	157 – 3080	0.48 – 9.42	[9,51]	2 – 299	0.01 0.91	[9,50]
MDMA	218 – 11986	1.13 – 62.02	[10,50]	46.3 – 1063	0.24 – 5.50	[50]
Ketamine	6 – 55136	0.03 – 231.93	[52]	280 – 820	1.18 – 3.45	[53]

The following step was to evaluate the analytical performance of the approach with 10-fold diluted oral fluid samples (**Fig. 5**). First, oral fluid was collected by passive drool or spitting into a tube. Thereafter, oral fluid was spiked with the corresponding

amount of each illicit drug at a time to deliver diluted oral fluid samples in the concentrations from 1, 2.5, 5, 10, 15, 20, 30, and 40 µM depending on the analysis of the illicit drug (**Fig. 5a**). This means that original oral fluid samples from a suspect consumer would contain levels of the drug of abuse in the range of 10 to 400 µM to be detected by the electrochemical sensor. This range falls in the reported physiological levels of illicit drugs in oral fluid (**Table 2**), although these levels might fluctuate depending on the time after last consumption, route of administration, the difference in dose, and the physiology of the subject [42]. Thereafter, the analytical performance was assessed by interrogating the diluted oral fluid after 15 min of the time of adsorption (**Fig. 5**) to assure that the illicit drug is completely adsorbed (**Fig. S8**). During these tests, Cl-PVP was excluded as the oxidation potentials of this drug fall in the potential window of an oral fluid interferent (between 0.5 – 0.7 V). Besides, a lack of stability of synthetic cathinones might be expected in oral fluid, making it difficult for its quantification [54]. **Fig. 5b-e** exhibit the SWV dynamic curve for cocaine, heroin, MDMA, and ketamine upon increasing concentrations, respectively. **Fig. S12** displays the raw SWV curves showing the necessity for the background-corrected data treatment in some cases as some differences might raise from batch to batch of the SPEs. Subsequently, the I_p of each SWV curve was displayed according to each concentration to evaluate the linearity of the sensor. **Fig. 5f-i** show the corresponding calibration curve for cocaine, heroin, MDMA, and ketamine, respectively. Cocaine calibration curve presented a LR from 2.5 to 20 µM at E_p= 0.89 V, with a sensitivity of 0.25 µA μM^{-1}, and a LOD of 1.2 µM (**Fig. 5f**). Heroin calibration curve exhibited a LR from 10 to 40 µM at E_p= 0.96 V, with a sensitivity of 0.07 µA μM^{-1}, and a LOD of 2.4 µM (**Fig. 5g**). MDMA calibration curve showed a LR from 2.5 to 20 µM at E_p= 1.03 V, with a sensitivity of 0.2 µA μM^{-1}, and a LOD of 1.0 µM (**Fig. 5h**). Lastly, ketamine calibration curve exhibited a LR from 5 to 40 µM at E_p= 0.93 V, with a sensitivity of 0.06 µA μM^{-1}, and a LOD of 2.6 µM (**Fig. 5i**). Besides, **Fig 5j-m** display an excellent intra-reproducibility in diluted oral fluid: at 10 µM for cocaine (RSD=2.1%, N=4); at 10 µM for heroin (RSD=5.3%, N=4); at 10 µM for MDMA (RSD=2.9%, N=4), and at 20 µM for ketamine (RSD=1.8%, N=4).

Importantly, a slight peak potential shift is observed when dealing with oral fluid samples instead of buffer, highlighting the importance of the analysis of complex matrices when dealing with the development of analytical devices. This shift can be

ascribed to a potential local pH change due to the oxidation of interferent species in the oral fluid matrix (e.g. current oxidation process at 0.5 V – 0.7 V). Moreover, the decrease in the I_p from the oxidation of the illicit drugs might be attributed to the protein adsorption on the electrode's surface (i.e. biofouling) which might displace the SDS and block the electroactive surface area. Despite these observations, the SDS/SPE system allows for the detection of illicit drugs in diluted oral fluid with a simple dilution step and with an affordable cost per analysis. Hence, this method opens new promises for the use of electrochemical sensors at roadside testing to identify criminal situations or at workplace scenes. Interestingly, the integration of a tailor-made script to enhance peak separation could provide the identification of several illicit drugs in the same sample (i.e. multidrug consumption) by its oxidation potential. This script has been successfully applied for the detection of ketamine [41] and heroin [38] in mixtures of illicit drugs and cutting agents. When there is a peak overlap due to similar oxidation potentials between several molecules, the script deconvolutes the peaks allowing for proper identification by their oxidation potentials.

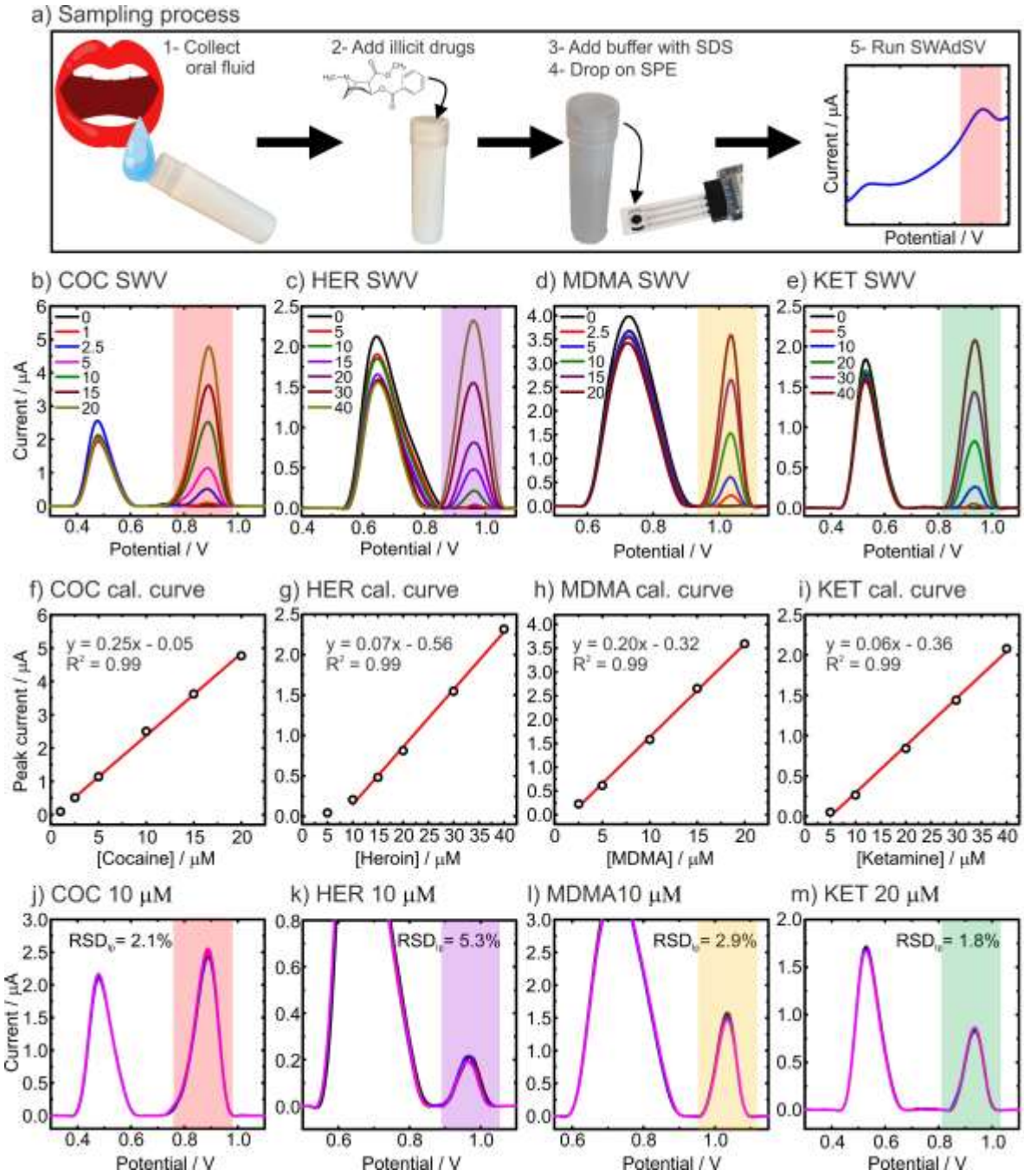

Fig. 5. *Analytical performance of the sensing concept at SDS/SPE using diluted oral fluid: a) Sampling method consisting of 1- oral fluid collection, 2-spiking, 3-dilution, 4-drop cast on SPE, and 5-SWAdSV test. SWVs of increasing concentrations from 1 to 40 µM for b) cocaine; c) heroin, d) MDMA, and e) ketamine. Corresponding calibration curves for f) cocaine; g) heroin, h) MDMA, and i) ketamine. Intra-day reproducibility for j) cocaine; k) heroin, l) MDMA, and m) ketamine; N=4. SDS concentration at 0.2 mg mL^{-1}. The tests were executed using a 10-fold dilution of spiked oral fluid with BR buffer at pH 9, pH 6, pH 5, and pH 8 for cocaine, heroin, MDMA, and ketamine, respectively. COC=cocaine; HER=heroine; KET=ketamine.*

4. Conclusions

This work demonstrates for the first time a simple and affordable method for the sensitive determination of illicit drugs in oral fluid (i.e. cocaine, heroin, MDMA, Cl-PVP, ketamine) by using a surfactant-mediated electrochemical analysis at SPE. Particularly, SDS is adsorbed at the carbon electrode's surface and yields the adsorption of illicit drug molecules, allowing for a higher electrochemical output by using SWAdSV. The accumulation of the illicit drugs at the surface of the SPE is suggested to occur: (i) due to the interaction of the lipophilic domains with the graphitic surface, and (ii) from the protonated form of the target molecules with the negatively charged heads of the SDS. First, the system was characterized by CV, EIS, and ATR-FTIR to evaluate the adsorption of SDS molecules. Thereafter, the adsorption-controlled phenomena at the SDS/SPE of the illicit drugs were assessed. After the optimization of the pH and the time of adsorption, the analytical performance of each illicit drug was presented reaching low micromolar levels. Finally, the application of this approach for the analysis in oral fluid samples was accomplished by a simple dilution step. A careful characterization of the oral fluid matrix and the analytical performance of the illicit drugs spiked in this matrix are detailed. Overall, the electrochemical method provides a simple strategy to detect illicit drugs on an unmodified SPE exhibiting similar analytical parameters to other electrochemical sensors with complex modifications. The next steps include: (i) the assessment of common interferences from biological fluids and cutting agents from drug doses; (ii) functionalization of the SPE to improve LOD and decrease biofouling; and last but not least (iii) the design of a sampling method that facilitates the use of the SDS/SPE system in the field. Overall, the new sensing concept holds significant promises for the development of miniaturized and portable electrochemical devices for rapid oral fluid analysis in roadside testing.

CRediT authorship contribution statement

Marc Parrilla: Conceptualization, methodology, investigation, data curation, writing - original draft, writing – review and editing. **Florine Joosten**: investigation, data curation, review and editing **Karolien De Wael:** Investigation, resources, project administration, writing - review and editing.

Declaration of Competing Interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version.

PDF file includes: Tables showing CV parameters, EIS fitting parameters, and peak current and peak potential values from SDS/SPE method; Figures displaying chemical structures of illicit drugs, optimization of the surfactant, FTIR spectra, the electrochemical study of SDS-mediated analysis, pH screening, plots from the scan rate study, time of adsorption study, SWV from saliva matrix effect, interferent study, and raw SWV profiles from calibration curve in diluted oral fluid.

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Highlights

- An enhanced voltammetric response of illicit drugs using surfactant-mediated solutions is explored.
- The adsorption of illicit drugs at a screen-printed electrode's surface is characterized.
- Square-wave adsorptive stripping voltammetry is described for micromolar detection of cocaine, heroin, MDMA, Cl-PVP and ketamine.
- An affordable method for the detection of illicit drugs is exemplified in oral fluid.

Graphical abstract