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Electrochemical Detection of Cocaine in Authentic Oral Fluid †

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Abstract: Illicit drug consumption remains a problem to public safety and health, with abuse of illicit drugs having increased significantly over the last years. A concern related to this abuse is driving under the influence of drugs (DUID). Currently, police and law enforcement agencies rely on the use of lateral flow immunoassays (LFAs), which suffer from a lack of specificity. In this report, we present a rapid, sensitive, and affordable electrochemical method for the detection of cocaine in oral fluid (OF) by square-wave adsorptive stripping voltammetry on screen-printed electrodes (SPE). For the first time, the effects of the OF matrix on the electrochemical sensing of cocaine are deeply explored. The interference of endogenous compounds in OF, cutting agents and adulterants is studied. Interestingly, the electrochemical signal for cocaine is shown to be partially suppressed by the biofouling properties of albumin and most probably other proteins present in the OF matrix. Thus, strategies to mitigate these biofouling properties are explored. Subsequently, two sampling methods for OF, expectoration and the use of a commercial OF collection device (i.e., the Intercept i2), are investigated. The developed method shows promising potential in point-of-care testing for recent illicit drug use.

Keywords: square wave voltammetry; oral fluid testing; cocaine; screen printed electrodes; forensic analysis

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1. Introduction

Even during the ongoing COVID-19 pandemic, the consumption of illicit drugs has remained a problem to public health and safety [1]. In the last decade, the number of worldwide drug users has grown at a 30% rate to reach 270 million users in 2018 [2]. In the same year, the highest number of cocaine seizures in Europe was reported [3]. An increasing concern related to the use of illicit drugs and cocaine is that of driving under the influence of drugs (DUID) [4]. In the large-scale European Union (EU) study "Driving under the Influence of Drugs, Alcohol and Medicines (DRUID)" (2012), it has been reported that the detection rate of illicit drugs in the general driving population was 1.9% [5]. This detection rate was higher in seriously injured drivers (2.3–12.6%). The World Health Organization (WHO) has estimated that over 39,600 traffic deaths were caused by DUID in 2013 [4]. Of these deaths, 14% were attributed to the use of cocaine. These numbers show that it is paramount to tackle the DUID issue to improve road safety. A potential solution is to perform more roadside tests to identify and block DUID.

The standard process of illicit drug detection in OF consists of two steps [6]. First, a presumptive test is performed on-site. If the results of this test are positive, they need to be confirmed in the laboratory using techniques such as gas chromatography or liquid chromatography coupled to mass spectrometry (GC- or LC-MS). For presumptive tests, lateral flow immunoassays (LFAs) are currently the gold standard even though they might exhibit some drawbacks as follows: (i) cross-reactivity with similar drugs; (ii) lack of

specificity; (iii) time consuming (>5 min); (iv) high-cost; and (v) short shelf lives due to the use of bioreceptors [7,8].

This work aims to tackle the identified issues related to the detection of cocaine in OF by the development of a rapid, affordable, and sensitive sensing method based on electrochemical sensors. The workflow of the sensing method is presented in Figure 1. For the first time, the OF matrix effects on the electrochemical sensing of cocaine are deeply explored by using screen-printed electrodes (SPE). First, the electrochemical behavior of cocaine in buffer solution is investigated by square-wave adsorptive stripping voltammetry (SWAdSV) which adsorption is enabled by the use of a surfactant. Second, the interference of endogenous compounds in OF and cutting agents and adulterants is studied. Interestingly, the electrochemical signal for cocaine is shown to be partially suppressed by the biofouling properties of albumin and most probably other proteins present in the OF matrix. Hence, strategies to mitigate these biofouling properties are explored. Subsequently, two sampling methods for OF, expectoration and the use of a commercial OF collection device (i.e., the Intercept i2), are investigated. Finally, the developed methodology is used to analyze authentic OF spiked with cocaine.

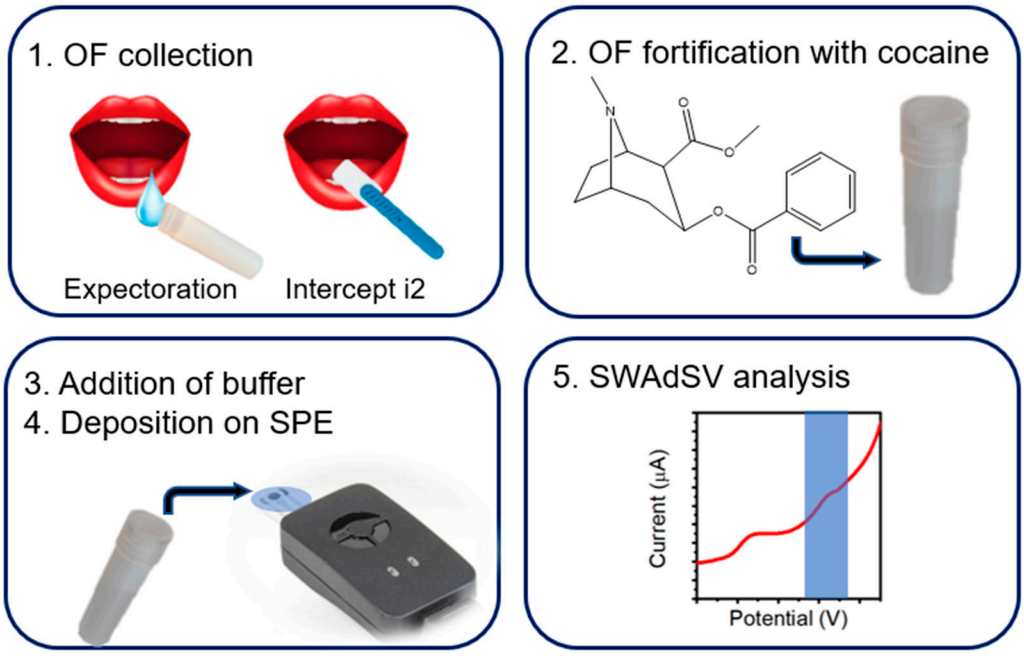

Figure 1. Workflow of the electrochemical sensing method for cocaine in OF. (1) OF collection via expectoration or using the Intercept i2 OF collection device, (2) OF fortification with cocaine, (3) dilution of the OF sample in buffer, (4) deposition of the sample on the electrode, and (5) SWAdSV test and analysis. OF = oral fluid; SPE = screen-printed electrode; SWAdSV = square-wave adsorptive stripping voltammetry.

2. Materials and Methods

2.1. Materials

Standards of cocaine·HCl were purchased from Chiron AS, Norway. Analytical grade salts of potassium chloride, potassium phosphate, sodium phosphate, sodium borate, sodium acetate, potassium hydroxide and sodium hydroxide were purchased from Sigma-Aldrich (Overijse, Belgium). Sodium dodecyl sulphate (SDS) was purchased from Sigma-Aldrich (Overijse, Belgium). Intercept i2 (OraSure Technologies) oral fluid collection devices were purchased from Qarad (Geel, 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 measurements were performed using a MultiPalmSens 4 (PalmSens, Houten, The Netherlands), a MultiEmstat3 (PalmSens, Houten, The Netherlands), or a PalmSens4 (PalmSens, Houten, The Netherlands) combined with a MUX8-R2 multiplexer (PalmSens, Houten, The Netherlands) controlled by PSTrace/MultiTrace software. Unmodified Italsens IS-C SPE (PalmSens, Houten, The Netherlands) were used for all experiments. The IS-C SPE contains a carbon working electrode (Ø = 3 mm), a carbon counter electrode, and a silver reference electrode. All potentials reported in this work are versus Ag pseudoreference electrode. Square wave voltammetric (SWV) measurements were performed in Britton–Robinson buffer with 0.1 M KCl by depositing 100 µL of the sample solution on the SPE. The samples were allowed to interact with the electrode surface for five minutes before the measurements were started. Instrumental parameters were: 60 mV amplitude; 25 Hz frequency; 5 mV potential step. All SWVs obtained were baseline corrected using a mathematical algorithm "moving average" (peak width = 1) in PSTrace software to improve the resolution of the peaks over the background.

2.3. Cocaine Detection in Oral Fluid

OF samples were collected from healthy volunteers from the research group immediately before analysis. Samples were collected at least 2 h after food consumption or taking any medication. OF collection was performed in two manners: (i) by expectoration in a 3 mL testing tube, or (ii) by using an Intercept i2 oral fluid collection device (OraSure Technologies). The OF samples were diluted in Britton–Robinson buffer solution (pH 10, unless specified otherwise) containing SDS at the desired dilution factor before electrochemical interrogation.

3. Results and Discussion

3.1. Analytical Characterization of Cocaine in Buffer Solution

In the first step, the electrochemical behavior of cocaine in buffer solution (pH 9) was studied. As our group had previously shown that the use of the surfactant SDS can enhance the cocaine signal, SDS was added to the buffer solution (0.075 mg mL^{-1}) [9]. Thus, a cocaine oxidation peak was observed around +0.85 V. The performance of the detection method was assessed by executing a calibration curve (I_p (µA) = 1.41 × $c_{cocaine}$ (µM) − 0.88) with concentrations in the range from 0.1 to 10 µM (Figure 2). While the limit of detection (LOD) of 1.0 µM is not adequate for roadside drug testing, it is similar to LODs reported for the direct electrochemical detection of cocaine using SWV by other authors [10,11].

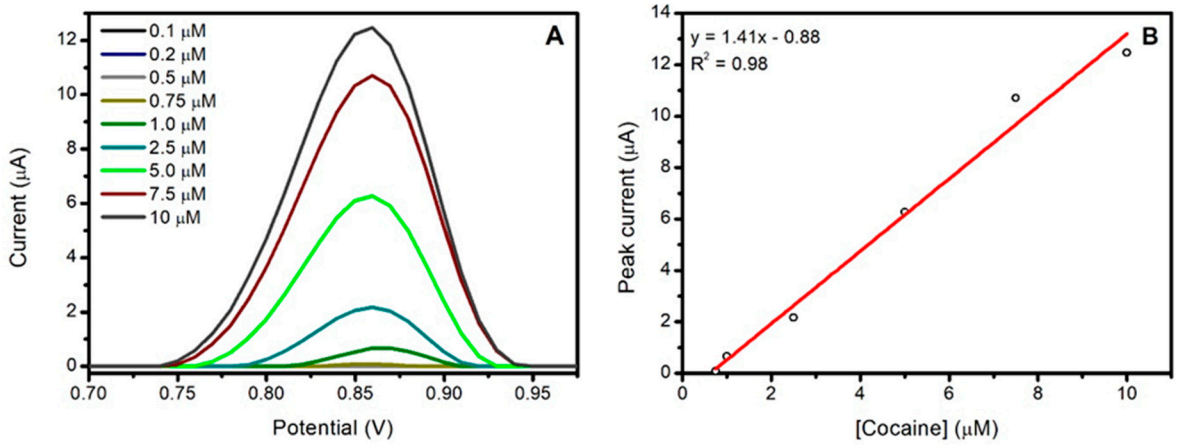

Figure 2. Analytical characterization of cocaine in buffer solution under optimal conditions. (**A**) SWVs of increasing cocaine concentrations from 0.1 µM to 10 µM, (**B**) corresponding calibration curve.

3.2. Study of the OF Matrix Effects

Before the electrochemical method was tested in OF, the influence of several compounds present in OF on the cocaine signal was investigated. Whole saliva is a complex heterogeneous mixture containing proteins, electrolytes and small organic compounds and is rich in antioxidants [12,13]. The effect of the anti-oxidants uric acid (200 µM) and ascorbate (vitamin C, 5 µM), as well as that of urea (4.5 mM) in a binary mixture with 5 µM cocaine in a buffer solution containing 0.075 mg mL^{-1} SDS was evaluated using SWAdSV. The concentrations of the potential interferents were selected according to the regular physiological levels found in OF [14]. The voltammograms showed that ascorbate and urea were not electroactive under the experimental conditions (Figure 3A). The voltammogram of uric acid showed an oxidation peak at +0.12 V, with a shoulder around +0.3 V. The presence of all three compounds resulted in a decrease in peak current for cocaine as compared to a 5 µM cocaine reference sample. This decrease was highest for urea, with a 21% loss in peak current.

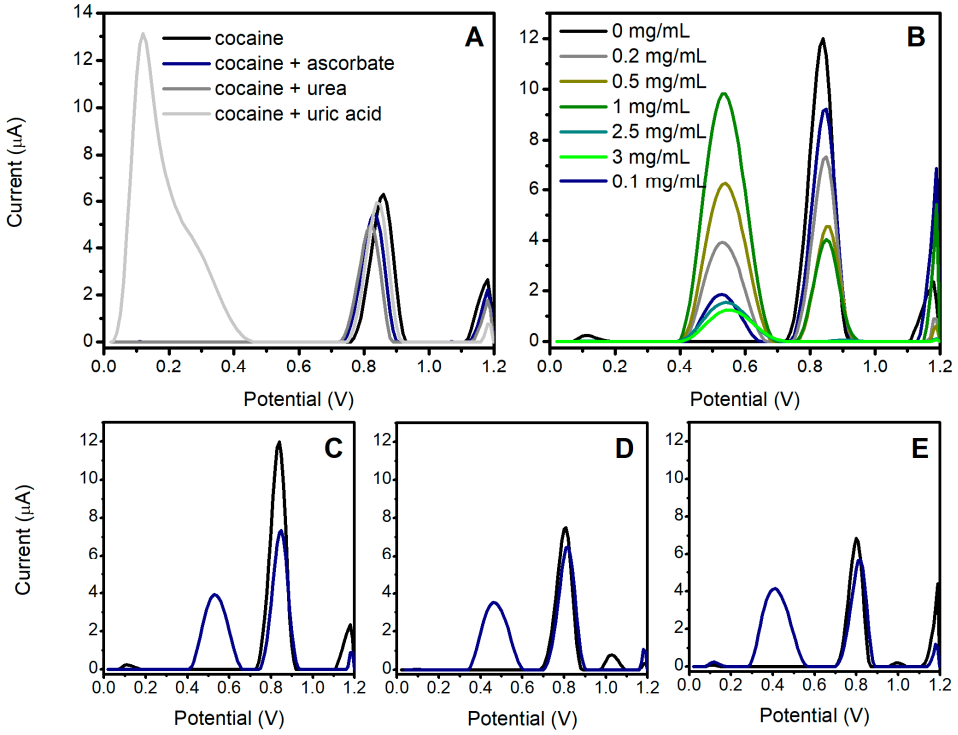

Figure 3. Investigation of the OF matrix effects on 10 µM cocaine under optimal conditions: (**A**) Effect of constituents: ascorbate, urea, uric acid and (**B**) effect of albumin concentration. Effect of the pH on the oxidation signal for 10 µM cocaine in the presence of 0.2 mg mL^{-1} albumin with (**C**) pH 9, (**D**) pH 10, and (**E**) pH 11. Black line: 10 µM cocaine, blue line: 10 µM cocaine in the presence of 0.2 mg mL^{-1} albumin. All tests were executed in BR buffer containing 0.075 mg mL^{-1} SDS.

Proteins are known to have biofouling effects on electrodes due to non-specific adsorption on the surface of the biochemical sensor [15]. This can result in the decrease in performance of the biosensor and loss in sensitivity and specificity for the target analyte. As saliva can contain over 1000 different peptides and proteins, the detection of cocaine in OF is expected to be hindered by biofouling effects [16]. To investigate this biofouling phenomenon, albumin was selected as a model protein because it is the most abundant protein in biofluids [17]. First, the effect of different albumin concentrations (0, 0.1, 0.2, 0.5, 1, 2.5, and 3 mg mL^{-1}) on the electrochemical signal for 10 µM cocaine was evaluated (Figure 3B). Albumin was shown to be electroactive, with an oxidation peak at +0.54 V. Interestingly, the albumin peak decreased at concentrations above 2.5 mg mL^{-1}. As expected, the cocaine peak current decreased with an increase in albumin concentration.

Unfortunately, at an albumin concentration of 3 mg mL^{-1}, the cocaine peak was completely suppressed. However, average levels of albumin in OF are ca. 0.9 mg mL^{-1} [18], which should still allow for the electrochemical detection of cocaine.

In a strategy to minimize the biofouling effects, the effect of pH was studied (Figure 3C–E). As the pKa values of the α-amino hydrogen of amino acids are in the range 8.72–10.70 [19], it was predicted that at pH 11 albumin would be negatively charged. Therefore, a repulsion by the negatively charged SDS moieties is expected. The SDS/SPE was tested with 10 µM cocaine and a binary mixture of 10 µM cocaine with 0.2 mg mL^{-1} albumin in buffer solutions of pH 9, pH 10, and pH 11. The albumin peak potential shifted towards less positive values with increased pH, as its deprotonated form is easier to oxidize. While the decrease in the electrochemical signal was 1.6-fold at pH 9, it was only 1.2-fold for pH 10 and pH 11. This indicated that less albumin was adsorbed at the electrode surface as predicted. It is important to note that the concentration of albumin and other proteins in OF might vary between individuals. To obtain a more reproducible method of cocaine detection, it is therefore important to minimize the biofouling effects of proteins. Hence, pH 10 was selected as a compromise situation between reduced biofouling effects of albumin compared to buffer pH 9, and a higher peak current compared to buffer pH 11.

3.3. Investigation of Two OF Collection Methods

Direct electrochemical measurement in OF is possible, as OF contains electrolytes and is ionically conductive [20]. However, since the OF composition and pH of individuals may vary, it is preferable to add a buffer solution in order to control the chemical composition and pH of the solution. Dilution of the buffer solution has the disadvantage that the concentration of the illicit drug in the total solution decreases, but it can cope with strong interferences. A dilution factor of 1:5 (OF:buffer) was selected as a compromise between the decrease in cocaine concentration, decrease in background effects, and stability of sample pH.

As OF collection by expectoration is slow and unpleasant for donors, and also suffers from a lack of hygiene, OF collection by a commercial device was explored. The Intercept i2 was used as a model device. The Intercept i2 works by placing it under the tongue of the donor until the indicator turns blue and the desired amount of is collected. According to the manufacturer, this device collects 1 mL of in an average time of 3–4 min [21]. To test the performance of the devices, several experiments were carried out to determine (i) the time of OF collection, (ii) the amount of OF collected, (iii) the amount of OF extracted, and (iv) the recovery of cocaine. First, the amount of collected and the collection time were examined. The OF from two individuals was collected three times using the Intercept i2. Before and immediately after collection, the devices were weighed. On average, 1.2 g of OF was collected. Assuming a density of 1 g mL^{-1}, this amounts to approximately 1.2 mL of OF, which is more than the manufacturer claimed. The average collection time was just over 1 min, which is substantially shorter than the waiting time mentioned by the manufacturer.

Three different approaches were explored for the recovery of cocaine and extraction of OF from the Intercept devices. OF was collected by expectoration and spiked with 10 µM cocaine. A small amount of spiked OF was put aside for comparison, while the remaining sample was collected with the Intercept device. The recovery of cocaine was examined by comparing the voltammograms obtained from the recovered samples with a reference voltammogram from the OF that was set aside (Figure 4A–C). In these experiments, the recovered fluids were diluted with 2 mL of buffer solution containing 0.075 mg mL^{-1} SDS (dilution factor 1:2). It was assumed that 1 mL of OF was collected with the Intercept devices, as this is the amount stated by the manufacturer and what laboratories work within their analyses. In the first cocaine recovery approach, the preservation liquid in the Intercept i2 collection vial was removed and replaced with the buffer solution. After the OF was collected with the Intercept device, the collection pad was placed in the collection vial. The vial was vigorously shaken, and then left to rest for 5 min so that diffusion

could take place, before the liquids in the collection vial were collected and analyzed with SWV. After recovery from the Intercept device, the peak currents for cocaine and albumin decreased 2.4-fold and 2.1-fold, respectively (Figure 4A). This indicated that the OF and cocaine recovery from the device was not complete. The second approach of recovery consisted of centrifugation at 3000 rpm for 5 min, as this is recommended in the manual by the manufacturer. After centrifugation, buffer solution was added to the recovered fluids. The voltammogram of the recovered cocaine showed a more intense peak than the reference voltammogram (Figure 4B). This could have been due to evaporation of the sample during the manipulation leading to an error in comparison to spiked OF. Recovery by centrifugation has the disadvantage that it makes the total procedure for roadside testing more difficult and more expensive. Therefore, as an alternative approach, the recovery was performed by pressing the collection pad using a syringe. To do this, the Intercept i2 collection device was broken open and the collection pad was removed. The recovered fluids were collected in a tube and mixed with the buffer solution. The voltammograms showed a slight decrease (9%) in peak current for cocaine (Figure 4C). The peak current for albumin decreased with 26%, indicating that albumin might be more retained at the collection pad than cocaine. As the change in peak current for cocaine was smallest when the recovery was performed using a syringe, this strategy was chosen as an optimal procedure for cocaine recovery. The recovery of cocaine in this approach was tested using three Intercept devices (Figure 4D–F). On average, 57.8 ± 4.8%) of the collected OF was recovered. For all three collection devices, the peak current of the recovered cocaine was higher than the peak current for cocaine in the reference sample. The increase in peak current was largest for device 1 (1.5-fold increase), which was also the device from which most oral fluid was recovered.

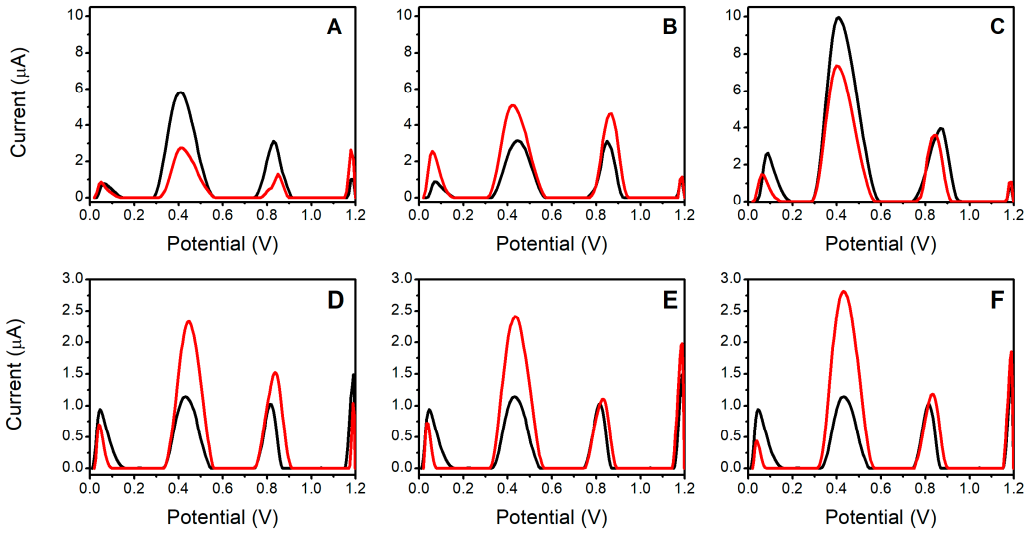

Figure 4. Recovery of 10 µM cocaine from the Intercept i2 collection device by (**A**) vigorously shaking, (**B**) centrifugation for 5 min at 3000 rpm, and (**C**) removing the collection pad from the device and pressing it using a syringe. Recovery of 10 µM cocaine from three Intercept i2 devices using the syringe method for (**D**) device 1, (**E**) device 2, and (**F**) device 3. Black line: 10 µM cocaine in OF for reference, red line: recovery of 10 µM cocaine from the Intercept i2 device. All SWVs for the recovery study were performed with 3-fold diluted OF in Britton–Robinson buffer pH 10 containing 0.075 mg mL^{-1}.

4. Conclusions

In this work, a rapid, inexpensive, and sensitive electrochemical method for the detection of cocaine in OF was explored. In buffer solution, the LOD for cocaine detection was found to be 1 µM. For the first time, the interference of endogenous compounds present in the OF matrix on the electrochemical detection of cocaine was studied. Albumin showed

to have fouling effects on the electrode, causing a decrease in the sensitivity. The antifouling effects were successfully reduced by adjusting the pH of the buffer solution from pH 9 to 10. A sampling method for the direct measurement in OF was developed and integrated with the SDS/SPE system, as a first step towards the application of electrochemical methods for illicit drugs detection in OF in the field.

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