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Ultrasensitive covalently-linked Aptasensor for cocaine detection based on electrolytes-induced repulsion/attraction of colloids

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Abstract

A quick and easy colorimetric sensor based on gold nanoparticles (GNPs) and aptamers for the detection of cocaine was developed. The sensor was named as 'GAPTA' and showed extremely interesting results regarding cocaine detection with a sensitivity to doses of 0.2 nM. The experimental approach consisted of creating a conjugate between GNPs (10 nm size) and aptamers as a sensing base with the addition of an electrolyte (NaCl) that plays the role of aggregation inducer. In the absence of the aptamer, the electrolyte was able to induce aggregation of the GNPs turning the color of the solution from red to blue while the presence of the aptamer is able to hinder the charges attraction and protects the GNPs from aggregating. The optimization of the aptamer and electrolyte concentration was determined to be 118 nM and 55 mM, respectively, and the resultant GAPTA sensor had a detection limit of 0.97 nM. Furthermore, the selectivity of the platform was tested in the presence of different interferents and showed a specific response towards cocaine while interference ranged between 20 and 40%. The applicability of the GAPTA biosensor was tested on synthetic saliva and demonstrated a sensitivity range between 0.2 and 25 nM. These results suggest the potential of the current colorimetric sensor in abuse drugs screening and creates a stable base for new routine platforms for biomedical and toxicology applications.

Keywords Aptasensor · Gold nanoparticles · Aptamer · Cocaine · Drug detection

1 Introduction

Substance abuse has been denoted as a serious public health disorder and cocaine is one of the top contenders for the major causes of addiction. Public health authorities have been working relentlessly with medical and toxicology sectors to develop new detection tools to lower drug traffic and substance abuse (Asturias-Arribas et al. 2011). Cocaine is a potent stimulant of the central nervous system inducing a euphoric feeling to the users (Shin et al. 2016) and the continuous abuse results in a serious relapsing disorder accompanied by important side effects such as anxiety, heart problem, immunologic issues and organ damage (Abnous et al. 2016).

Until now, various analytical techniques have been used for cocaine detection. Chromatographic detection (HPLC, LC-MS, and GC–MS), as well as immunoassay-based tools (RIA, ELISA), have been extensively used as the main approach for drug abuse detection (Aleksa et al. 2012; Lee et al. 2013). These techniques are reproducible and perform really well with excellent sensitivity. Nonetheless, these methods require highly trained personnel, expensive instruments, complicated steps and elongated times which can be a big issue when needing quick access for analysis results.

In the past decade, electroanalytical methods emerged as a preferable alternative for biosensor technology. Electrochemical-based biosensors have many of the advantages sought by the public authorities and medical sectors alike for drug abuse detection including cost-effectiveness, portability, flexibility, ease to use and the high sensitivity which compared to standard approaches (Asturias-Arribas et al. 2013). In this regard, researches are in constant work

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to propose novel devices and techniques based on natural and/or synthetic molecules for drug abuse detection.

Aptamer-based cocaine detection in combination with different optical methods (colorimetry, fluorescence, surface-enhanced Raman scattering (SERS) is an interesting alternative to conventionally used methods. Aptamers are short and single-stranded oligonucleotides molecules that have been integrated into biosensing technologies as a recognition molecule with a high affinity to target molecules (Hou et al. 2014; Li et al. 2016; Na et al. 2015; Taghdisi et al. 2015). In addition to their good affinity, they have low immunogenicity, stable, low-cost, and easy to synthesize. Aptamers could be engineered for a variety of targets ranging from small-sized molecules to entire cells. Given their advantageous properties and unique features, they can be valuable components in the development of highly performant sensing platforms that satisfy the analytical expectations and the market necessities(Bai et al. 2014; Bozokalfa et al. 2016; Golub et al. 2009; Huang et al. 2013; Roushani and Shahdost-Fard 2015).

In this work, we propose a colorimetric assay for a quick, easy, and label-free cocaine screening based on the use of colloidal gold nanoparticles (GNP) conjugated to a specific 32-bp aptamer (that we named it as 'GAPTA' sensor). The GNP-aptamer complex is shown to keep its stability in a highly concentrated salt solution due to the negative surface charges of the aptamers. Furthermore, the complex was put under aggregating conditions with salt after adding cocaine. This aggregation induces a shift in surface plasmon resonance (SPR) that could be seen through the color change.

GNPs are known to possess an attractive/repulsive behavior in the presence of electrolytes. This feature was exploited for the preparation of the current sensing platform. The ideal concentrations of salts and aptamers were optimized experimentally for cocaine detection. Afterward, different cocaine analogs were tested in the same way to demonstrate the sensitivity and selectivity of the prepared platform. Lastly, the applicability of the current unit was further validated by testing its capacity for cocaine detection in saliva containing cocaine.

2 Experimental section

2.1 Chemicals and reagents

Cocaine aptamer (32 base pair: 5′-C6–NH2–AGACAAGG AAAATCCTTCAATGAAGTGGGTCG–SH2–3′) was purchased from Metabion International AG (Steinkircihen, Germany). Gold (III) chloride ($HAuCl_4$) and sodium citrate were purchased from Sigma-Aldrich (St. Louis, USA). All reagents used are of analytical grade and were purchased from Sigma Aldrich unless mentioned otherwise.

2.2 GNPs synthesis

Colloidal gold nanoparticles were synthesized based on the inversed Turkevich method (Turkevich et al. 1951) following the protocol described before (Babaei Afrapoli et al. 2018). It is based on the adjustment of the GNPs size through the modulation of the sodium citrate levels. An inverse linearity is observed between the GNPs size and the amount of citrate in the reaction. The detailed procedure is included in the Supplementary information. The size of the prepared GNPs was analyzed using a standard protocol of scanning electron microscopic (SEM) imaging.

2.3 GNP-aptamer conjugation

Before the preparation of the biosensor, GNP-aptamer conjugates were prepared according to the procedure described in the supplementary information section. The obtained GNPs-aptamer conjugates as well as the attachment of cocaine were analyzed using dynamic light scattering (DLS) (zeta-sizer Nano ZS, Malvern Instruments Ltd., UK) to measure the particles' size change for each step of the sensor preparation. The tested samples were the synthesized GNPs, GNP-aptamer conjugates, and GNP-aptamer-cocaine. Both particle size and zeta potential were measured for all samples.

2.4 GNP-aptamer sensor construction

The GNP-aptamer complex for the colorimetric cocaine sensor (which will be then named as 'GAPTA') was prepared to operate as described in Fig. 1. In a 96-well plate, GNP (100 μL) with or without aptamer (20 μL) solutions were deposited and followed by the addition of NaCl solution (20 μL). The plate was agitated at room temperature for 20 min (120 rpm) and the absorbance spectrum of the reaction mixture was obtained between 350 and 800 nm (Biotek plate reader). Color variations of the GNP were normalized at 520/620 nm (Mei et al. 2013). All analyses were executed in triplicates and the same conditions were maintained for all subsequent experiments.

2.5 Optimization

The optimization procedure to determine NaCl and aptamer concentrations, the size of GNPs to be used, and the stability of the reaction for data acquisition for the optimal performance of the GAPTA sensor was performed in the same experimental conditions and the optimal conditions are as follow: 55 mM for NaCl, 118 nM for the aptamer, GNPs size was 10 nm, and the reaction time was set to 60 min. The detailed experimental procedures and results are included in the Supporting Information section (Fig. S1-S3).

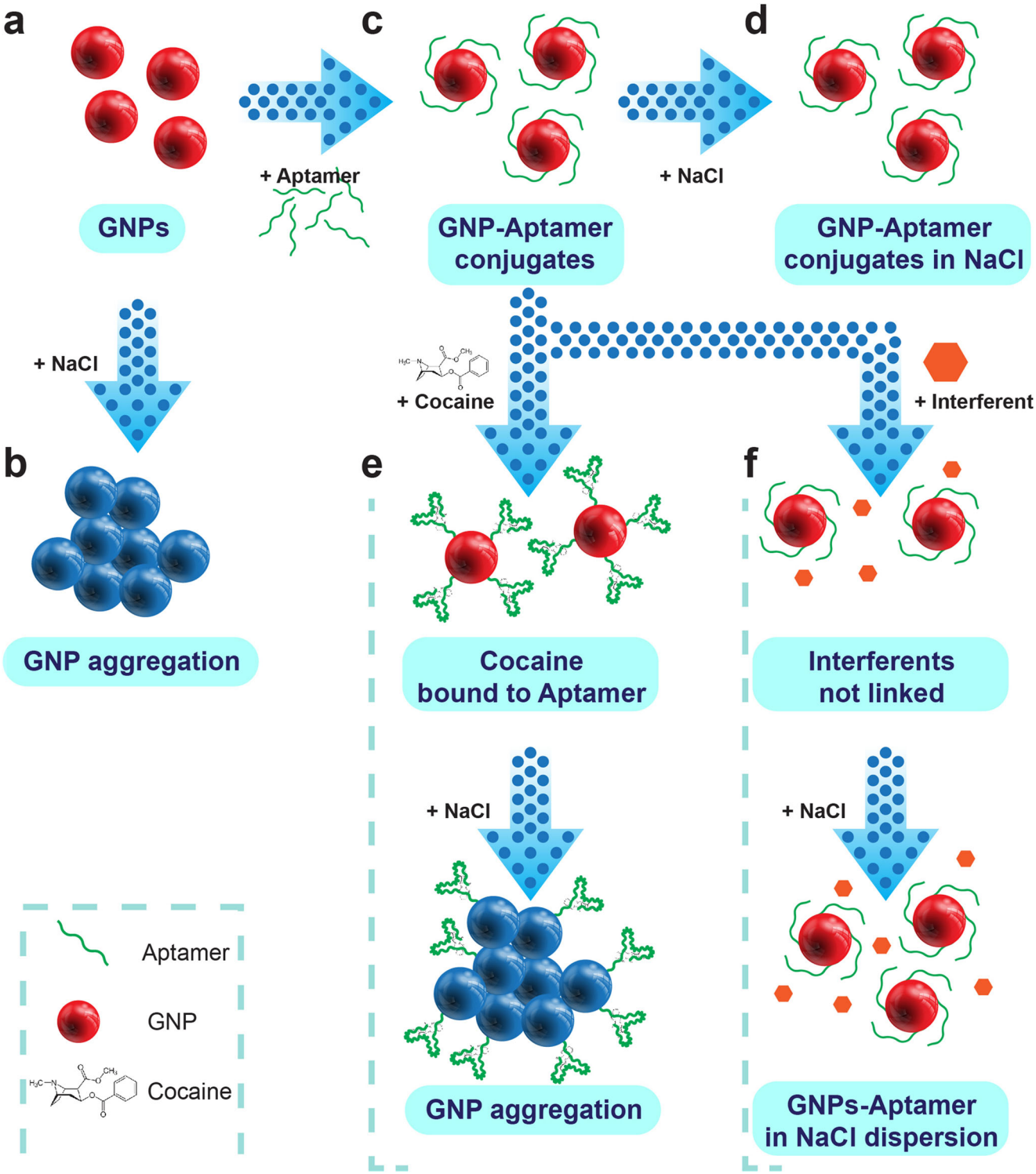

Fig. 1 Illustration of the mechanism of action of the GAPTA sensor. The addition of NaCl to a solution of GNPs results in an aggregation of the gold colloids and a change of color from red to a blue purple color (**a**, **b**). In the case of functionalized GNPs with aptamer (**c**), the addition of NaCl is not able to induce aggregation (**d**). However, GNP-aptamers will selectively link cocaine and change conformation which allows the aggregation in the presence of NaCl (**e**). This phenomenon is not observed with interferents or other abuse drug molecules (**f**)

2.6 Characterization of cocaine GAPTA sensor

In order to determine the sensitivity and selectivity of the GAPTA sensor, the platform was prepared according to the details described earlier to which different concentrations of cocaine were added.

Sensitivity 100 μL of GNP followed by 20 μL of the aptamer (118 μM) were deposited in the wells and incubated for 20 min under agitation. Afterwards, 30 μL of different cocaine concentrations were added at final concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10, 15, 20, 25, 30 nM. Phosphate buffer (20 mM, pH 7.4) was used as a negative control. Another 20 min at 120 rpm incubation was performed and then, 20 μL of NaCl (55 mM) was added to the mixture and the corrected optical densities (620/520 nm) were acquired. The results were used for tracing a standard curve to check the linearity of the GAPTA sensor.

Selectivity The colorimetric GAPTA sensor selectivity was tested in the presence of different cocaine analogs considered as potential interferents. 30 μL of 10 nM amphetamine, methamphetamine, codeine, morphine, and JWH-073 were added to the GNP-aptamer mixture and OD were acquired after following the same experimental procedure for the testing as described before. Phosphate buffer was used as negative control while cocaine (10 nM) was used as a positive control.

The stability of the GAPTA sensor was analyzed in function of time and the procedure and the results are included in the Supplementary information section (Fig. S4).

2.7 Sample application

Synthetic saliva was prepared according to a standard recipe for the matrix (Demir et al. 2016). Briefly, two sets of tests were prepared where synthetic saliva was spiked either with 10 nM or 20 nM of cocaine. The detection procedure was done following the experimental procedure detailed when validating the sensor's sensitivity. Normalized OD was obtained and concentrations were calculated using the linear standard curve obtained from the different concentrations of cocaine. The experiment was repeated three times. In addition, to explore the effect of protein present in biological samples as an interfering agent during cocaine testing. Different concentrations of BSA (1.0–10 nM) were tested alone or in the presence of 10 nM of cocaine and tested according to the procedure described above.

3 Results and discussion

3.1 Characterization of the GNP-aptamer conjugate

The synthesized GNPs, and GNP-aptamer conjugates used in the current sensor were analyzed using dynamic light scattering (DLS) for size and surface charge measurement. The bare GNPs and GNP-aptamer were found to have an average size of 8.29 ± 2.18 nm (PDI: 0.586) and 13.64 ± 4.52 nm (PDI: 0.427), respectively. In addition, GNP-aptamer-cocaine gave a size of 16.86 ± 4.45 nm (PDI: 0.490). The increase of the particles size was predictable due the attachment of the aptamer on the GNP surface. Analyzing the conjugate size is an important step for the surface characterization of the current sensor. Moreover, the charge over the surface of the conjugates via the measurement of the zeta-potential is an important factor in the determination of the conjugation efficiency. Indeed, the surface charge results showed −26.83 ± 2.87 mV, −2.04 ± 1.41 mV, and − 19.83 ± 3.2 mV for the bare GNPs, GNP-aptamer, and GNP-aptamer-cocaine, respectively. The data obtained confirms the expectations on the conjugates formation. The covalent conjugation of the aptamer on the surface of GNPs via –SH groups will cover the surface and lower their charge which eventually will hinder their interaction with the electrolytes (Fig. 1c). on the other hand, the aptmers will have a change in conformation after interaction with cocaine and thus, the GNPs surface is partially uncovered to interact with the electrolytes (Fig.1e). It has been shown that cocaine aptamer functionalized with –SH groups are able to covalently attach with GNPs (Guler et al. 2017).

3.2 Cocaine analysis via GAPTA sensor

The principle behind cocaine sensing by the colorimetric GAPTA sensor is described in Fig. 1. In this regard, we can see different cases on how the sensor will function according to the elements present. Non-aggregated GNPs show a red color when freely present in the solution (Fig. 1a) which is due to the joint undulation of the free electrons of the GNPs (Wang and Sun 2006). When electrolytic salts are added to the GNP solution, it induced the aggregation of the colloids by decreasing the distance between the nanoparticles via the disruption of the repulsion between the negatively charged molecule and consequently, adjacent GNPs are prone to interact (Li and Rothberg 2004). The aggregation of the GNPs changes the color of the solution for red into blue/purple color due to various types of interaction such as van der Waals (Fig. 1b). On the other hand, the addition of the aptamer to GNPs will permit their complexation via van der Waals interactions (Fig. 1c) and hinders the effect of NaCl on the GNPs consequently inhibiting the aggregation effect (Fig. 1d) (Li and Rothberg 2004; Mei et al. 2013).

When cocaine is added to the mixture, we observe a change of color from red to blue meaning the happening of the colloids aggregation (Fig. 1e). This is due to the high affinity of the aptamer to cocaine which induces a conformational change to the aptamer resulting in its release from the GNPs (Lee et al. 2018; Li and Rothberg 2004; Xu et al. 2018).

However, when interferents are added in the mixture, no aggregation of GNPs was observed and the solution stayed red-colored even when NaCl was added (Fig. 1f). This is explained by the specificity of the aptamer to cocaine and that the aptamer protected the GNPs from the electrolyte-induced aggregation. The optimization procedure resulted in selecting 10 nm-sized GNPs, 55 mM of NaCl, 118 nM of aptamer, and a 1 h incubation period for the last reading. Detailed results can be seen in the Supplementary Information.

3.3 Sensor characterization and cocaine sensing performance

Sensitivity The validation of the prepared colorimetric GAPTA sensor was performed through the analysis of the sensitivity and the selectivity of the platform. A dose range of 0.2–30 nM of cocaine was tested and the corresponding visual observations, absorbance spectrum, and normalized OD were taken (Fig. 2). Using phosphate buffer as a negative control showed expected results comparable to the one described in the optimization process in the absence of cocaine. Additionally, the shift in absorbance from 520 to 620 nm was observed in a dose-dependent manner of cocaine (Fig. 2a). The normalized OD of the cocaine GAPTA sensor showed a linear increase between 0.2–25 nM of cocaine where it started curving to a plateau by the time reaching 30 nM. As such, the proportional linearity of the sensor to cocaine levels was extremely satisfying ($y = 0.02x + 0.32$; $R^2 = 0.997$) (Fig. 2b). The limit of detection was calculated using the 3Sb/m formula giving a result of LOD; 0.97 nM. The repeatability of the test gave a standard deviation value (SD) of ±0.0094 and a coefficient of variance (c.v) equal to 1.85%. The observed results showed that the designed sensor allowed the detection of cocaine levels of as low as 0.2 nM. With the increase in cocaine levels, the OD increased with a discernable color change that could be observed by the naked eye.

Selectivity The demonstration of the GAPTA cocaine selectivity in the presence of other potential interferents (amphetamine, methamphetamine, codeine, morphine, and JWH-073) was performed experimentally and the normalized OD for cocaine showed by far the highest values compared to

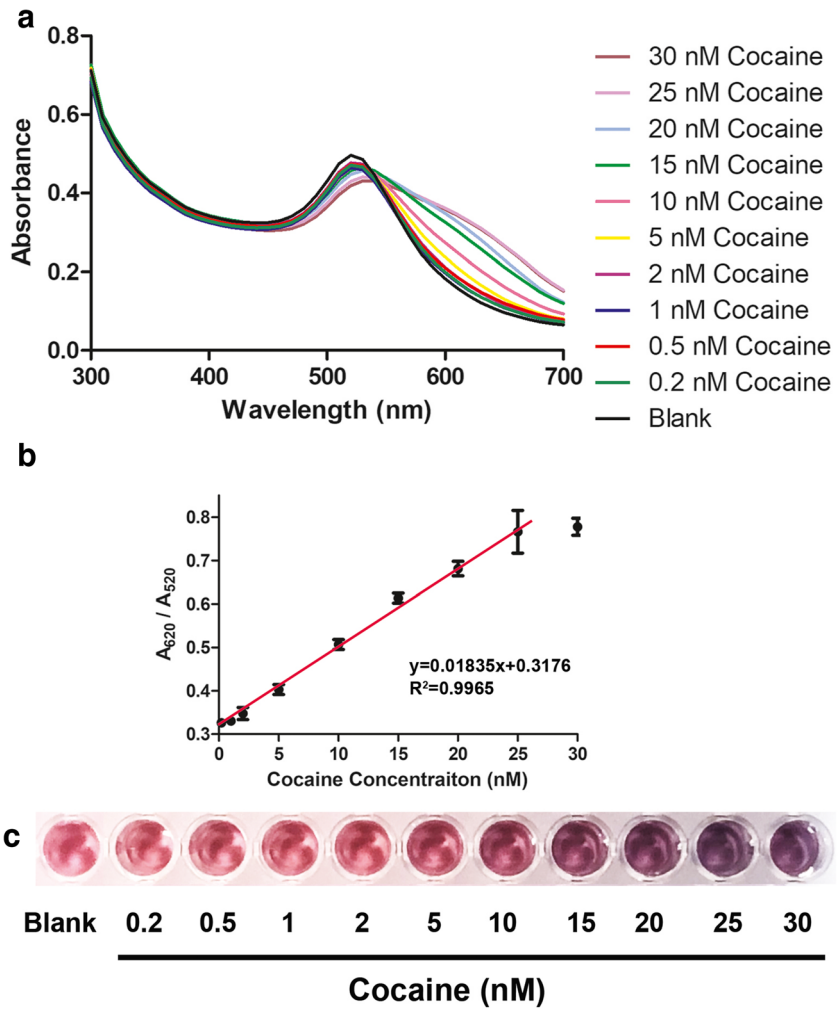

Fig. 2 Characterization of the GAPTA cocaine sensor. (**a**) UV-Vis scanning of the GAPTA sensor with different cocaine concentrations (0.2–30 nM). (**b**) The linearity of the sensor was observed between 0.2–25 nM ($R^2 = 0.99$) after which a plateau is observed. (**c**) Visual observations of the different concentrations tested in the plate

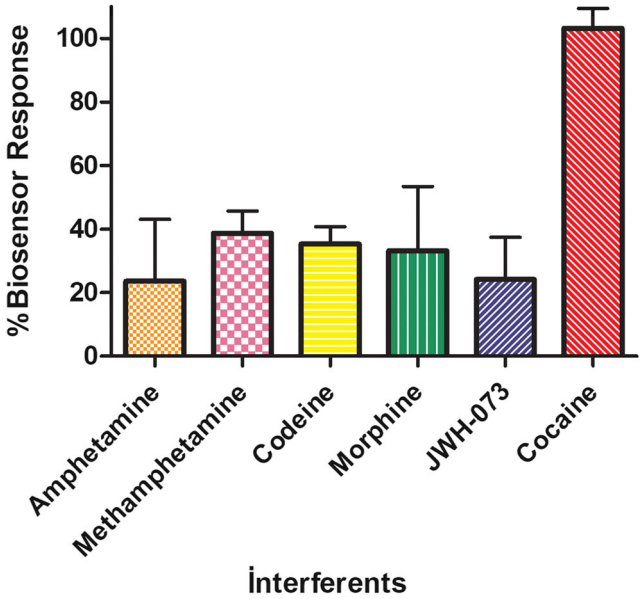

Fig. 3 Response of the GAPTA cocaine sensor in the presence of various interferents. Different analytes (10 nM) were tested as described in Material and Methods with 55 mM NaCl, 118 nM aptamer, and 10 nm-sized GNPs. The response was calculated considering cocaine values as 100% ($n = 3$) and the interferents levels reached between 20 and 40%

other molecules (Fig. 3). In contrast, the addition of 10 nM of each interferent was able to garner some response from the GAPTA sensor with large deviations in their values. The interferents results demonstrated various response levels ranging between 20 and 40% response. Because of the difference between these interferents and cocaine results being significant, there should be a possibility for false positive response and thus a finer tuning of the aptamer choice could solve the issue.

The designed GAPTA cocaine sensor was meant as a quick and easy approach for the optical screening of nanomolar levels of cocaine. This platform demonstrated a low visual LOD (0.97 nM) compared with other similar approaches using either colorimetric or fluorimetric-based GNP-aptamer complexes (Qiu et al. 2018; Smith et al. 2014; Zhang et al. 2008). In these works, a limit of detection for cocaine was found to be in the micromolar levels (summarized in Table 1) which emphasizes on the performance of our current GAPTA cocaine sensor that able to detect nanomolar amounts. Smith et al. prepared a colorimetric gold-aptasensor for cocaine detection using a phone application for result acquisition. They used single-stranded and double-stranded aptamers for cocaine detection and were able to reach a LOD of 15 μM for adsorbed aptamers (Smith et al. 2014) while on the other hand, Qiu et al. obtained a LOD equal to 10.5 μM (Qiu et al. 2018). Zhang et al. were able to obtain a more sensitive sensor with a lower LOD reaching 2.0 μM (Zhang et al. 2008).

It should be noted that the GAPTA cocaine sensor's sensitivity with its low LOD is comparable to other fluorescent-based or electrochemical transducers-based transducers (Baker et al. 2006; Grabar et al. 1995). Nonetheless, the current GAPTA cocaine sensor is not very performant when the samples are colored, thus, suggesting its application with clear matrices such as saliva. Its strong points are demonstrated in its quick and easy application satisfying the analytical requirements.

3.4 Cocaine analysis via GAPTA sensor

In the aim of testing the applicability of the current GAPTA cocaine sensor, we tested its detection efficiency on synthetic saliva spiked with two different concentrations of cocaine. Table 2 describes the results obtained during this experiment. Normalized OD of the sample was converted into

Table 1 Comparison of LOD from different aptamer-based sensors

Approach	Strategy	LOD	Reference
Colorimetric	Aptamer and GNPs	0.97 nM	Our work
Colorimetric	Aptamer and GNPs	2 μM	[24]
Colorimetric	Dye replacement	2–600 μM	[27]
Colorimetric	Thiolated DNA aptamers/GNPs	50–500 μM	[28]
Fluorescence	FRET*	10 μM	[29]
Fluorescence	Aptamer-based machine	5 μM	[30]
Fluorescence	Isothermal circular amplification	0.19 μM	[31]
Fluorescence	Rolling circle amplification	0.48 nM	[32]
Fluorescence	Dual probe of aptamer and hairpin	2 nM	[33]
Fluorescence	Aptamer fragment/fibre	0.16 μM	[34]
Electrochemistry	Rolling circle amplification	1.3 nM	[35]
Electrochemistry	Electron transfer	10 μM	[25]
Electrochemiluminescence	Silica NP amplification	1.3 pM	[36]

*FRET: Fluorescence resonance electron transfer

Table 2 Detection of cocaine levels in saliva

Spiked Amount (nM)	GAPTA sensor response (nM)	Recovery Level (%)
10	9.71 ± 0.67	97.05
20	21.33 ± 1.39	106.64

concentration using a standard curve of cocaine (as described in the sensitivity experiments) and for a 10 nM of cocaine spiked in the saliva, a value of 9.71 nM was given by the GAPTA sensor. For the second cocaine dose tested (20 nM), the GAPTA sensor gave a value of 21.33 nM. The statistic differences were found to be insignificant after several repetitions ($n = 3$).

Biological samples are generally composed of different molecules including proteins and other electrolytes that may interfere with the sensing ability of the biosensor. Blood, saliva, and urine are known to contain different amounts of proteins and salts (Lin and Chang 1989; Martin 2011; Neyraud et al. 2003). Bovine serum albumin (BSA) was used as a protein representative to explore the interference over the sensitivity of the current cocaine platform. BSA alone did not induce any apparent interference. However, the addition of BSA with cocaine induced a 64.5% loss in the estimation of cocaine (data not shown). This interference is believed to be due to a masking of the molecules by the BSA. It has been shown that BSA has an adsorptive behavior to gold surfaces of sensors using quartz crystal microbalance with dissipation monitoring (QCM-D) and surface plasmon resonance (SPR) techniques (Diaconu and Schäfer 2014). Indeed, the limitation of this study is the lack of actual sample measuring where the laboratory conditions are standardized and close to perfect conditions compared to what exists in the real biological samples. Though the selectivity and sensitivity of the current sensing platform have been described, there is a need to introduce intermediary sample preparation steps during applications with biological samples. Generally, it is a common practice to conduct sample preparation procedures prior to sensing applications. Depending on the target and the sensor type, the methods applied are various (Chan et al. 2012). Of interest to the current study, biological sample handling using protein precipitation and salt elimination techniques is warranted in order to take full advantage of the current sensor potential.

4 Conclusion

The current study described a label-free, easy and quick to use colorimetric GNPs-aptamer biosensor for the detection of cocaine at nanomolar levels. The functioning of this GAPTA cocaine sensor was confirmed through visual observations, spectroscopic measurements, and SEM imaging analysis. The sensitivity and selectivity were satisfactory with a linear detection in the nanomolar levels. Furthermore, the prepared sensor was able to be successfully applied to spiked saliva to detect cocaine. These results emphasize the potential of the GAPTA sensor's position as a contender against chromatographic and other instrumental analysis methods. The experimental procedure duration for cocaine testing using the GAPTA sensor is estimated to take less than 90 min which further highlights the importance of such new methods in drug abuse detection. Nonetheless, such colorimetric assay is still in its developing process and suffers from some disadvantages such as the non-usability over colored test samples. Therefore, more efforts are needed to enhance such techniques and enable them to reach routine applications.

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Availability of data and material all details are included in the supplementary information section.

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Compliance with ethical standards

Conflicts of interest/competing interests The authors declare that they have no conflict of interest.

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