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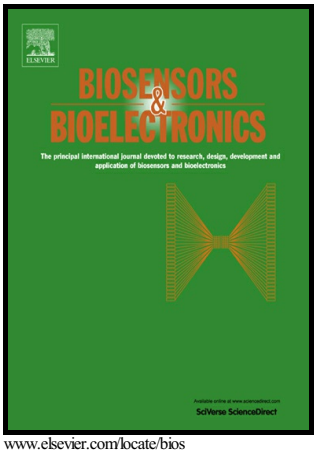

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Highly Sensitive and Specific On-Site Detection of Serum Cocaine by a Low Cost Aptasensor

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Abstract: Cocaine is one of the most used illegal recreational drugs. Developing an on-site test for cocaine use detection has been a focus of research effort, since it is essential to the control and legal action against drug abuse. Currently most of cocaine detection methods are time-consuming and require special or expensive equipment, and the detection often suffers from high cross-reactivity with cocaine metabolites and relative low sensitivity with the best limit of detection reported at sub nanomolar (nM) level. In this work, an aptasensor has been developed using capacitive monitoring of sensor surface incorporating alternating current electrokinetics effects to speed up molecular transport and minimize matrix effects. The aptasensor is rapid, low cost, highly sensitive and specific as well as simple-to-use for the detection of cocaine from serum. The assay has a sample-to-result time of 30 seconds, a limit of detection of 7.8 fM, and a linear response for cocaine ranging from 14.5fM to 14.5pM in standard buffer, which are great improvements from other reported cocaine sensors. Special buffer is used for serum cocaine detection, and a limit of detection of 13.4 fM is experimentally demonstrated for cocaine spiked in human serum (equivalent to 1.34 pM cocaine in neat serum). The specificity of the biosensor is also demonstrated with structurally similar chemicals, ecgonine ethyl ester and methylecgonidine. This biosensor shows high promise in detection of low levels of cocaine from complex matrices.

Keywords:

Capacitive biosensor, aptasensor, AC electroosmosis, point of need, cocaine

1. Introduction

Cocaine, also known as benzoylmethylecgonine, is a strong stimulant used mostly as an illegal recreational drug (Karila et al., 2010). Drug abuse is a serious global public health problem and a contributing factor to severe social problems. To control drug abuse problem and curtail its use, a reliable, simple to use and low cost cocaine test is highly sought after for on-site illegal drug screening by law enforcement and employment background check.

Conventional methods for cocaine detection are mostly mass spectrometry-based analytical techniques or high pressure liquid chromatography assays coupled with various detection methods such as UV-absorbance or fluorescence (Feng et al., 2014; Jiang et al., 2012; Hamaguchi et al. 2001). These methods have limits of detection (LOD) around nanomolar (nM) level, and they are often associate with shortcomings such as being time consuming, high cost, requires skilled personnel and complex samples preparation (Wen et al., 2011). Hence, there is an unmet need for the development of an easy and low cost method for on-site cocaine detection in complex biological fluids.

Recently, aptamer-based biosensors (also known as aptasensors) have received considerable attention for cocaine detection (Mokhtarzadeh et al., 2015; Hermann and Patel, 2000; Zhang et al., 2008). Aptamers are single-strands DNA or RNA oligonucleotides, and recognize specific targets owing to hydrogen bonding, electrostatic or hydrophobic interactions, etc. (Ellington and Szostak, 1990; Brody and Gold, 2000). Due to their high sensitivity, low cross-reactivity to small molecules and high stability, they are gaining popularity as a probe for biosensors based on specific binding (Citartan et al., 2012; Jayasena, 1999). Several types of aptasensors have been reported for cocaine detection. Qiu et al. used a hairpin-probe and isothermal circular strand-displacement amplification to increase the response of the cocaine capture probes (Qiu et al., 2013). This method showed a LOD of 190 nM. Zhang *et al.* developed a colorimetric aptasensor using gold nanoparticles (AuNPs) for cocaine detection as low as 2 μM in NaCl solution (Zhang et al., 2008). In a chemiluminescence aptasensor, AuNPs has been used to improve the LOD of cocaine detection to nM level in phosphate-buffered saline (PBS) (Li et al., 2011).

In addition to the aforementioned optical methods, aptasensors can also be adapted for electrochemical detection (Yunhui, 2010). Zhang et al. (2012) developed an aptasensor based on the formation of a supramolecular aptamer fragments/cocaine complex with an LOD of 0.1 μM.

Two fragments of anti-cocaine aptamer self-assembled into a supramolecular cocaine–aptamer fragments complex only in the presence of cocaine molecule. The cocaine was detected by the change of the electron transfer resistance, which was strongly correlated to cocaine concentration in PBS. Jiang et al. (2012) demonstrated a cocaine sensor using two engineered aptamers in connection to redox-recycling signal amplification. The electron transfers were enhanced by electrochemically depositing graphene/AuNP nanocomposites on a screen printed carbon electrode. The presence of the target cocaine and subsequently added biotin-modified secondary binding aptamers would form sandwich complexes on the electrode surface. The LOD of this assay was 1 nM cocaine. The aforementioned electrochemical-based aptasensors, although cheap and easy to implement, do require labelling which would make the detection process complicated and long, and might affect the bio-affinity between the aptamers and the target cocaine molecules (Liu et al., 2010).

In this report, we developed an easy-to-use, highly sensitive and specific cocaine aptasensor. The sensing method used here is known as AC electrokinetics (ACEK)-enhanced capacitive sensing (Li et al, 2015). This method operates by using a carefully-chosen AC signal to detect the interfacial capacitance change of aptamer-modified electrodes in a sample, and the AC sensing signal will also simultaneously induce enrichment of molecules by ACEK effects during the detection. For cocaine detection, low voltage AC signals applied over a meso-size interdigitated electrode (IDE) sensor is sufficient to induce AC electroosmotic (ACEO) effect (Mirzajani et al., 2016), which generates microflows around the electrodes convicting cocaine molecules towards the sensor, speeding up the binding of cocaine with capturing aptamer, thus shortening the response time to 30 s. Its LOD is 7.8 fM cocaine which is very sensitive comparing with other reported methods (Table 1).

For point-of-need detection applications, it will be more relevant to test cocaine in biological samples such as serum. Testing complex matrices has always been challenging for biosensors, since there exist many other large bioparticles at high concentrations in serum. For ACEK microfluidics, another ACEK effect, dielectrophoresis (DEP), will also be induced by the applied AC electrical field. As DEP is more pronounced with larger molecules, ACEK's application in small molecule detection is therefore hampered by unwanted accumulation of larger molecules such as proteins in serum. In a previous work detecting bisphenol A in serum, high dilution factor (1:1,000) was used to achieve the needed specificity (Lin et al. 2017). While

dilution works when detecting targets at high concentrations, it reduces the sensor's ability to detect low concentration small molecules in serum. In this report, the ACEK capacitive assay overcomes the interferences from high concentration proteins by adding proteinase to serum samples and achieves an LOD of 13.39 fM cocaine. Moreover, this aptasensor utilizes a simple protocol and does not require signal amplification or trained technician to operate, which makes it very suitable for point of use scenario.

2. Sensing mechanism and sensor structure

2.1 Interfacial capacitance sensing

ACEK capacitive sensing method adopts IDEs as its sensor, which consists of a series of parallel microband electrodes with alternating microbands connected to a common pad. The IDEs are functionalized with probe molecule to achieve detection specificity. Only target molecules are expected to bind with probe molecules immobilized on the IDE surface. The binding events are detected by tracking the capacitance change at the interface between the electrode and sample solution. This method has been used to detect small molecules, proteins, virus particles, and nuclei acids. (Cui et al., 2015; Liu et al., 2017; Cheng et al., 2017a; Cheng et al., 2017b).

As schematically shown in Fig. 1, binding of cocaine molecules with the immobilized aptamer causes an increase in the thickness of the dielectric layer at the IDE surface. Before surface functionalization, the bare electrode exhibits an interfacial capacitance due to the formation of electrical double layer (EDL) with a Debye length of λ_{edl}, which is electrically equivalent to having a dielectric layer with a thickness of λ_{edl} (Fig. 1 (a)). After the immobilization of the aptamer and the blocking molecules on the surface (Fig. 1 (b)), another layer λ_0 will be added to λ_{edl}. Then, the dielectric layer thickness becomes $\lambda_0 + \lambda_{edl}$ and the interfacial capacitance of the sensor before the binding of target molecule is expressed as

$$C_{int\_0} = A_{int} \,/\, (\frac{1}{\varepsilon p}\lambda_0 + \frac{1}{\varepsilon s}\,\lambda_{edl}), \qquad (1)$$

where A_{int} is the surface area of the interfacial capacitor of the functionalized electrode, ε_p is the probe molecule permittivity and ε_s is the double layer permittivity. When cocaine molecules bind to the immobilized probe, a third dielectric layer with a thickness λ_c will be added to λ_{edl} and λ_0(Fig. 1 (c)). The interfacial capacitance expression becomes

$$C_{int\_1} = A_{int,co} \,/\, \left(\frac{1}{\varepsilon p}(\lambda_0 + \lambda_{coca}) + \frac{1}{\varepsilon s}\,\lambda_{edl}\right) \quad (2)$$

Based on equations 1 and 2, the binding of Cocaine molecules can be detected by calculating the capacitance change ratio: $(C_{int\_1} - C_{int\_0})/ C_{int\_0}$.

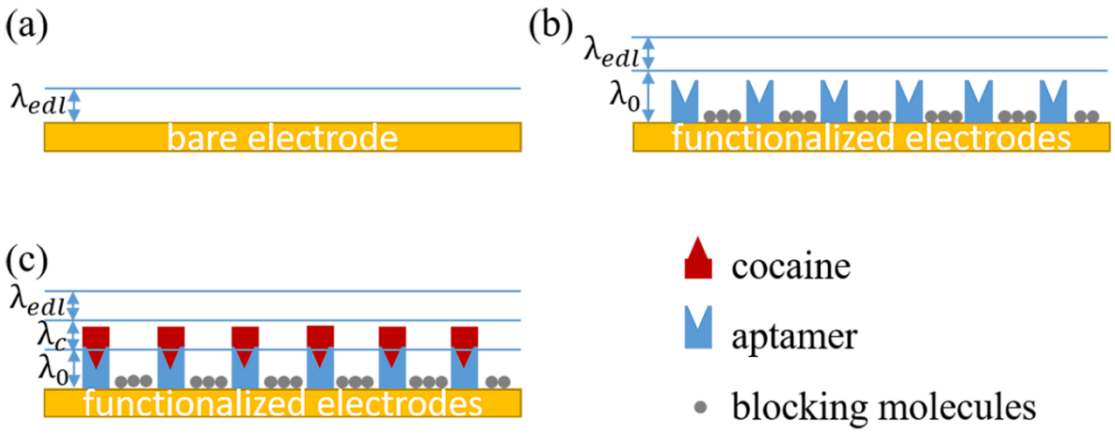

Fig. 1. *Changes in the interfacial capacitance of an electrode at different stages of sensor functionalization and cocaine binding. (a) Before functionalization, λ_{edl} is the thickness of the EDL (b) After immobilization of the aptamer, and the thickness of interfacial dielectrics becomes $\lambda_{edl} + \lambda_0$. (c) Cocaine molecules bind to the immobilized aptamer and the dielectric thickness increases by λ_c , which causes a decrease in the interfacial capacitance value.*

2.2. AC electroosmosis for accelerated aptamer-Cocaine binding:

Due to molecular diffusion of target analytes, affinity-based biosensors often suffer from lengthy binding time and lack of desirable sensitivity (Sheehan and Whitman, 2005). To overcome this limitation, particles can be manipulated using AC electrokinetic microfluidics with microelectrodes. There are three major ACEK effects, dielectrophoresis (DEP), AC electroosmosis (ACEO) and AC electrothermal (ACET) effects. With AC electric field inducing particle and fluid movement, biomolecules can be accumulated in-situ over the surface of electrodes (Wu 2008).

In this work, ACEO microflows are expected to be more effective than DEP for cocaine enrichment due to cocaine's small size (Liu et al., 2014; Castellano et al., 2013). As conceptually shown in Fig. 2, a non-uniform AC electric field induces counter-ions, which will move under

the electric fields tangential to the IDE surface, generating swirls-like ACEO flows above the IDEs. Those flows carry the target (cocaine) towards the IDE surface, which speeds up the transport of target molecules to the electrode surface and bind with the immobilized probe. The relation between ACEO fluid velocity and the applied electric field is given by the following equation,

$$u_{ACEO} = -\frac{\varepsilon_m}{\eta} \cdot \Delta\xi \cdot E_t \tag{3}$$

where ε_m and η are the permittivity and viscosity of the medium, E_t is the component of the electric field strength tangential to the electrode surface, and $\Delta\xi$ is the voltage drop over the interfacial layer including the EDL and molecular deposition at the electrode surface (Castellanos et al., 2003).

As discussed above, ACEO effect can induce microfluidic vortices above electrodes to accelerate the transport of cocaine molecules to the electrode's surface for binding (Wu et al., 2005), which significantly improves the detection sensitivity and response time. To promote ACEO effect, the test samples are diluted in electrolytes with low to medium ionic strength. As a result, the interface at the sensor/solution will exhibit a strong capacitive property with a high electric impedance. Therefore, a significant portion of the applied voltage will drop across the interface to produce ACEO flows (Yang and Wu 2008). Further, meso-size IDE arrays were adopted here based on the findings that large IDEs favor the generation of ACEK microflows to DEP effect (Cui et al, 2016). To maximize the enrichment effect by ACEO, we further optimized the AC frequency of sensing signal.

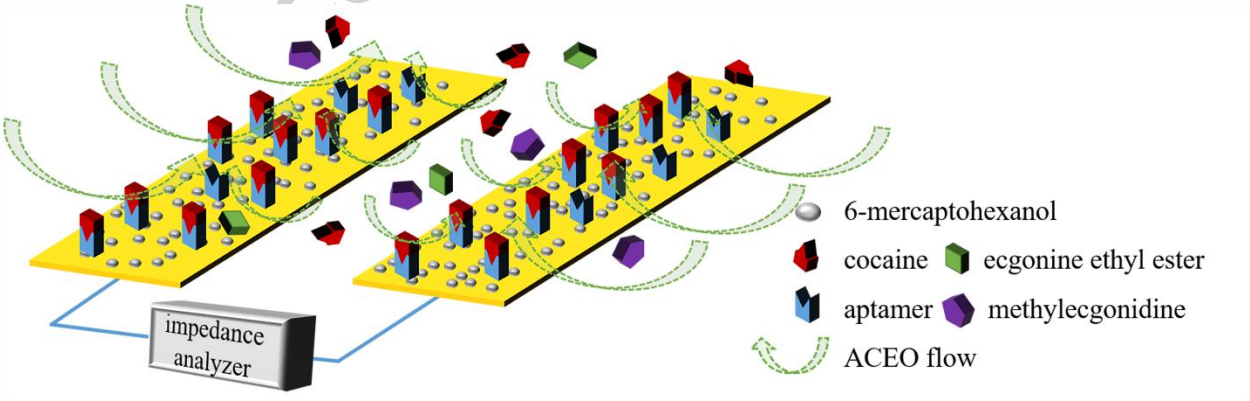

Fig. 2. *(a) Excitation of ACEO effect is integrated into capacitance measurement during a cocaine detection experiment. The ACEO flows carry cocaine moleculess toward functionalized sites and speed*

up the sensor response. The aptamer will bind with cocaine with its chemical structure shown as (b), but not with (c) ecgonine ethyl ester and (d) methylecgonidine

2.3 Circuit network of the electrode-fluid system

As aforementioned, the sensor used for this test is an IDE array. Its impedance, when immersed in liquid, can be electrically represented by an equivalent network of capacitors and resistors. The circuit, as conceptually shown in Fig. 3(a), includes each electrode digit's resistance (R_{wire}), the charge transfer resistance (*Rct*) and the interfacial capacitance originated from electrode-fluid interface (C_{int}). There are also fluid resistance and dielectric capacitance between the electrode digits, which are respectively represented by R_s and C_s. This cocaine test is based on non-Faradaic affinity sensing (there is no DC current flows across the interface), and the binding will be detected by tracking the interfacial capacitance. At the assay frequency, the electrical current between an electrode pair predominately takes the path formed by C_{int} and R_s (Li et al., 2013). Hence, the equivalent electric network could be simplified as a series connection of two interfacial capacitances (C_{int}) and an electrolyte resistance R_s (Cui et al., 2013a).

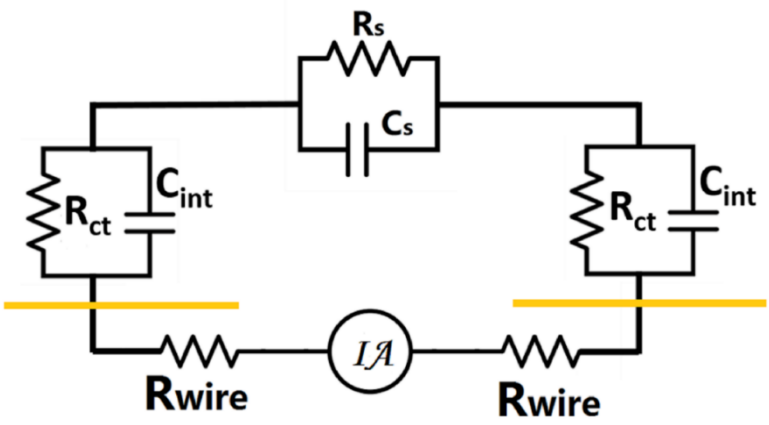

Fig. 3. *Equivalent circuit of the electrode sensor with sample fluid.*

A fitting of the electrodes' impedance spectrum is needed to extract the RC resonant frequency for inducing ACEO flows. The sensor impedance was measured over a frequency range of 40–110 MHz with the testing buffer placed over the IDE. (The impedance spectrum is given in the supplementary material.) Based on the fitting of the IDE's impedance spectrum (also

shown in the supplementary material), the values for each circuit element in Fig. 3 were obtained as follows. $Rwire = 8\,\Omega, Cint = 153\,nF, Rct = 47\,\Omega, Rs = 948\,\Omega, and\ Cs = 51.8\,pF$. Therefore the optimal frequency for ACEO effect is estimated to be around 1kHz, based on the following equation (Cheng et al. 2016),

$$F_{opt} = \frac{1}{(2\pi \mathrm{Rs\ Cint})} = \frac{1}{(2\pi * 948 * 153 * 10^{-9})} = 1097.29\ \mathrm{Hz} \quad (3)$$

3. Materials and experimental procedures

3.1 Sample preparation

Chemical reagents were purchased from ThermoFisher (Grand Island, NY). Cocaine, ecgonine ethyl ester and methylecgonidine solutions were purchased from Sigma Aldrich (St. Louis, MO). The sample solution used for ACEK capacitive assay was based on 1: 100 dilution of 1x PBS (150 mM Na^+) in ultrapure water (denoted as 0.01x PBS).

The sequence of 5′ thiol modified cocaine specific aptamer is 5'-ATCTCGGGAGACAAGGAAAATCCTTCAATGAAGTGGGTCTCCC-3'(Liu and Lu 2005). After aptamer immobilization, 1.0 mM 6-mercaptohexanol in ultrapure water was used to block the electrodes. Cocaine hydrochloride solution (1.0 mg/mL in methanol) was diluted in 0.01x PBS to make stock solution. 0.01x PBS was used to serially dilute cocaine stock solution and make cocaine samples of 1 pM, 100 fM, and 10 fM in 0.01x PBS, so that the ionic strength of samples were kept constant during all the serial dilutions. To test cocaine levels in human, human serum was 1:100 diluted in pure water. Then, cocaine was spiked into the aforementioned sample solutions.

3.2 Sensor preparation and surface functionalization:

The detection of cocaine is performed using gold electrodes fabricated based on printed circuit board (PCB) technique with a simple and low cost process. A detailed description of the fabrication steps is provided in a previous work by Mirzajani et al. (Mirzajani et al., 2017). The electrode digit is 400 μm wide and 1cm long with a 200 μm spacing between each. The IDEs are electrically connected to two contact pads, which are then used to connect to the impedance analyzer.

Before immobilizing the aptamer on the surface, the electrodes were rigorously cleaned. The effectiveness of the cleaning procedure has been confirmed by significant decrease of the

interfacial capacitance at the solution/electrode boundary (Cui et al., 2013b). The first part of the cleaning process consists of immersing the electrode into 100 nM sodium hydroxide (NaOH) and connect it to a potentiostat. The electrode potential was swept from $-0.3\,V$ to $1.1\,V$. After 5 scans of cyclic voltammetry, the electrodes were rinsed with water. Then, the chips were soaked in NaOH in a glass beaker on a hot plate-stirrer for 15 min then in acetone for 10 min. Later, they were rinsed by isopropyl alcohol for 10 s and deionized water for another 10 s, and dried by an air gun. To make it hydrophilic, the electrode surface was treated by a UV ozone cleaner for 15 min. Then the electrode surface was ready to be functionalized.

Sensor functionalization includes immobilization of cocaine-specific probe and blocking the surface of the electrodes. The immobilization of the probe is performed by loading 10 μL of 10 μM cocaine-specific aptamer diluted in 0.01× PBS over the electrode and incubating it in a humidor for 24 hours. 1.0 mM 6-mercaptohexanol was used to block the surface. After 3 hours of blocking, the chips are ready for testing. Two types of control tests were carried out as well. One was testing the blank buffer solution (0.01× PBS) without target molecules by a functionalized sensor, and the other one was testing cocaine on dummy electrodes (electrodes that were blocked without probe immobilization), so as to gauge the magnitude of response artifacts caused by the applied electrical field.

3.3 Measurement and data processing

This work uses ACEO based capacitive sensing to monitor the probe / target binding. The interfacial capacitance was found by measuring the electrode's impedance at a fixed AC frequency and voltage continuously during the testing. Using a high precision impedance analyzer (Agilent® 4294A), the capacitance of the sensor was recorded through its LAN port and sampled 201 times for a duration of 30 s. Then, the percentage change of the measured capacitance per minute was found. The normalized capacitance change rate ($d|C|/dt$, where |C| means normalized capacitance) was calculated for each cocaine concentration by applying the least square linear fitting. Each concentration was tested five times with a new functionalized sensor each time.

4. Results and discussion

Based on our prior work on ACEK capacitive sensing of small molecules (Cheng et al, 2016; Lin et al., 2017), an AC signal was applied at a specific frequency (1 kHz) and amplitude (400 mV) directly to the sensor for 30 s to induce ACEO effect and measure the capacitance change in real time. The choice of the signal amplitude was not arbitrarily. In fact, high voltage amplitude strengthens the ACEO flow and could enhance the cocaine binding. However, higher voltages may damage the surface functionalization of the electrode by causing the immobilized molecules to detach, leading to sensor malfunction. Based our previous discussion, 1 kHz was chosen as our testing frequency.

4.1 Dose response and limit of detection (LOD)

To obtain the sensor's specific response to various concentrations of cocaine, 14.5 fM to 1.45 pM of cocaine in 0.01x PBS was tested using functionalized and dummy sensors respectively. The dummy sensors do not have specific aptamers immobilized on them. Their responses will reflect how much artifact was caused by the applied electrical field. Each cocaine sample was loaded into a chamber sealed over the electrode surface.

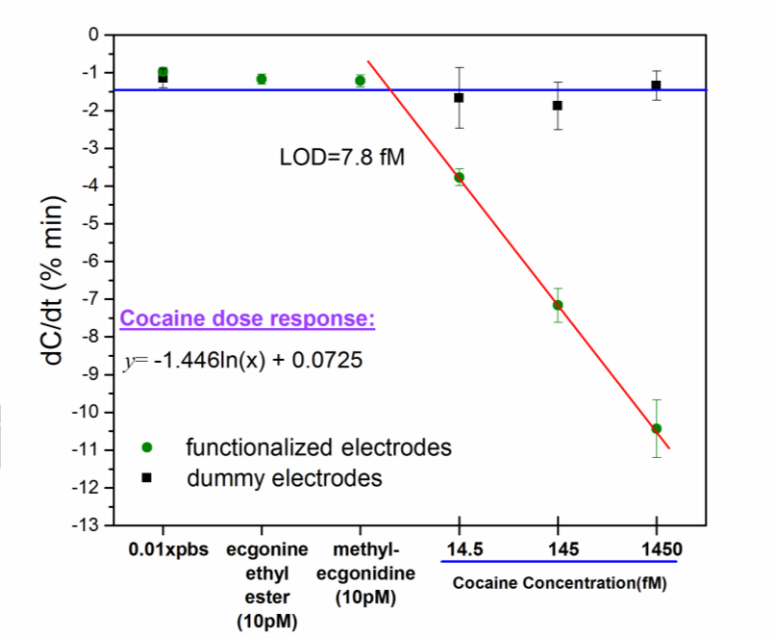

Fig. 4. *Response of cocaine in 0.01× PBS on both functionalized sensors and dummy sensors. The response of the functionalized sensors to the background (0.01x PBS) and the two structurally similar chemicals are also shown here, and their magnitudes are less than the cut-off value. Each data point is*

the average of five repeated tests. Error bar indicates standard deviation. The red slanted line is the extracted dose response.

As shown in Fig. 4(a), the normalized capacitance change rate increased with higher cocaine concentration. The $d|C|/dt$ value was $-3.77\ \%/min$ for 14.5 fM cocaine and reaches $-7.16\ \%/min$, and $-10.44\ \%/min$ for 145 fM and 1.45 pM of cocaine, respectively. The capacitance change rate for the blank control solution without cocaine was − 0.97 %/min, which is negligible in comparison to those triggered by cocaine in the samples. It is also important to notice that the responses for 14.5 fM to 1.45 pM of cocaine by the dummy sensors were no more than 1.92 %/min. These results confirm that the changes of C_{int} mainly come from the binding of cocaine molecules with the aptamer immobilized on the electrodes.

Based on Fig. 4, the sensitivity of the sensor was calculated to be $\frac{d|C|}{dt}(\%/\boldsymbol{min}) = \boldsymbol{-1.446 \cdot ln(x) - 0.0725}$, where x was cocaine concentration in fM. The sensor response is linear over the tested range from 14.5 fM to 1.45 pM. The cut-off response of the sensor is defines as 3 standard deviations from the response of background solution. Consequently, the cut-off value for sensor response ($\boldsymbol{d|C|/dt}$) was found to be $\boldsymbol{-1.22\ \%/min}$. By substituting this value into the dose response equation, the LOD was calculated to be 7.8 fM cocaine.

3.2 Selectivity:

For a biosensor to be practical, selectivity is one of the critical attributes that is highly desired for biosensors (Cheng et al., 2017b). Here, the specificity of cocaine sensor was tested against methylecgonidine and ecgonine ethyl ester, two chemicals that are structurally similar to cocaine. With cocaine-specific aptamer, the capacitance change rates for 10 pM of methylecgonidine and ecgonine ethyl ester were $\boldsymbol{-1.18 \pm 0.13\ \%/min}$ and $\boldsymbol{-1.22 \pm 0.16\ \%/min}$, respectively, as shown in Fig. 4(b). Obviously, the sensor's responses for both chemicals at 10 pM were more positive than the cut-off value for the sensor, thus considered as non-responsive.

3.3 Serum samples

The performance of this biosensor in a real life setting was evaluated by detecting cocaine in biological complex samples. Human blood serum was chosen for this test. First, the

effect of serum matrix was studied by testing 14.5 fM, 145 fM and 1.45 pM cocaine spiked in 1:100 diluted serum. The sensor responses for the background solution and interference molecules are found to be considerably larger for the spiked serum samples than those in 0.01x PBS. As shown in Figs. 5 (a) and (b), the sensor responses are $-2.04 \pm 0.16\ \%/min$ and $-2.36 \pm 0.68\ \%/min$ for diluted serum (the background) and 10 pM methylecgonidine (the negative control). The capacitance change rate of 14.5 fM cocaine spiked in serum became $-2.93 \pm 0.92\%\ /min$, and was no longer distinguishable from that of the background or the negative control values. Again, the $d|C|/dt$ of the cut-off response was found by subtracting 3 standard deviations (3x 0.16 $\%/min$) from the response of the background solution ($-2.036\ \%/min$). This value was then substituted in the dose response equation, $y1 = -1.318\ln(x) + 0.2518$ and the LOD was calculated to be 126 fM. This degradation in sensitivity can be explained by the deposition of interference molecules (proteins) in serum on the surface of the sensor.

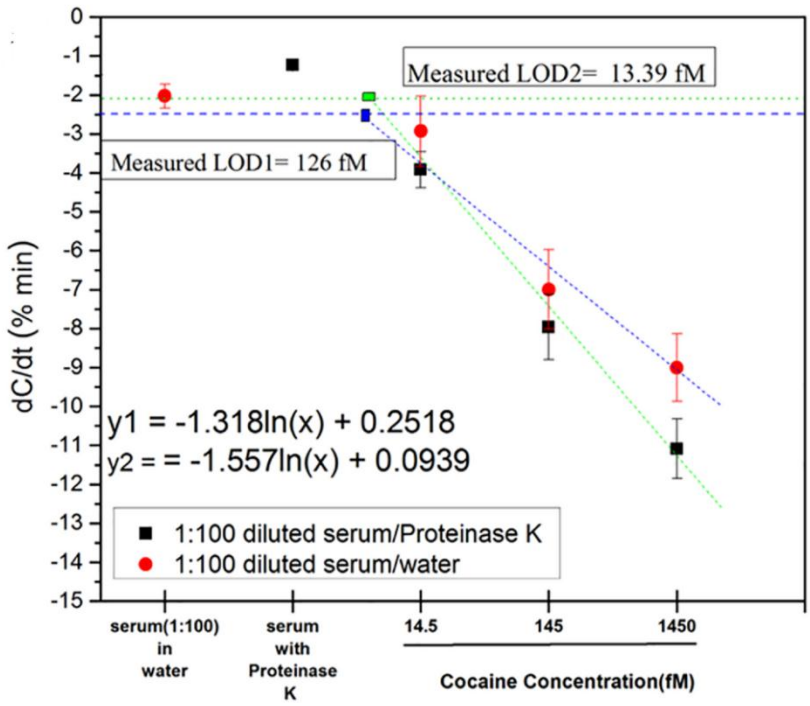

Fig. 5 *Sensor response to different concentrations of cocaine samples. The dotted line is the extracted standard curve. The sensor sensitivity is the slope of the extracted curve. The horizontal lines indicate the cut-off dC/dt values of the sensor response, which correspond to 126 fM when serum samples are diluted only in pure water and 13.39 fM cocaine when proteinase K is added to the testing buffer. Error bars represent standard deviation.*

We improved the testing buffer by adding proteinase into serum. Proteinase K can break down proteins in the serum (Maurizi, 1992). 10 mg/mL of Proteinase k was diluted 1:20 in 0.01x PBS. The neat serum was then 1:100 diluted in the aforementioned solution. After loading 10 µL of serum samples spiked with 14.5 fM to 1.45 pM cocaine, the $d|C|/dt$ magnitude was observed to gradually increase with increasing cocaine concentration. As shown in Fig. 5 (a) (with additional data in the supplementary material), the three cocaine serum samples and the background were clearly differentiated from each other. The responses were $-3.92 \pm 0.46\ \%/min$, $-7.95 \pm 0.82\ \%/min$, and $-11.08 \pm 0.40\ \%/min$ for a cocaine concentration of 14.5 fM, 145 fM and 1.45 pM, respectively. The dose response is linear from 14.5 fM to 1.45 pM, and can be expressed as $y2 = -1.557ln(x) + 0.0939$. For the background and 10 pM methylecgonidine, the $d|C|/dt$ values were $-|1.23 \pm 0.15\ \%/min$ and $-2.16 \pm 0.04\ \%/min$, which are smaller in magnitude than those in diluted serum without protease. The LOD was calculated to be 13.39 fM, equivalent to 1.34 pM in neat serum. A comparison of the responses between two types of buffers shows that adding proteinase K to the testing buffer has significantly improved the sensitivity of the sensor, and also decreased the responses from serum background.

To sum up state-of-the-art in cocaine aptasensors, Table 1 presents the figures-of-merit for the previously reported cocaine sensors discussed in Sec.1 and those of ACEK capacitive cocaine aptasensor. As indicated in the table, these approaches require complicated sample preparation (Zhang et al., 2008; Li et al., 2011), expensive equipment, and highly trained personnel to acquire and process the data (Qiu et al., 2013), which limits the usability of these sensors at point of need. It also should be noted that all methods (Table 1) tested cocaine in analytical buffer solutions. The ACEK capacitive aptasensor detects cocaine in serum samples and reaches an LOD of 1.39 fM. It is demonstrated to be a highly selective, rapid, low cost and simple to fabricate and operate aptasensor for cocaine detection, which makes it very suitable for point of care diagnosis.

Table 1: Comparison of sensor performance by electrochemical and optical cocaine aptasensors.

Detection method	Amplification/Labeling	Dynamic range	LOD	Reference

Fluorescence	Amplification: Isothermal circular strand-displacement amplification	0.2–100 mM	190 nM	Qiu et al. (2013)
Colorimetry	Label: AuNPs	20nM-200mM	2 μM	Zhang et al. (2008)
Chemiluminescence	Label: AuNPs	1–10nM	0.48 nM	Li et al. (2011)
Electrochemical impedance spectroscopy (EIS)	Supramolecular aptamer fragments/cocaine complex	0.1–20 μM	10 nM	Zhang et al. (2012)
Differential pulse voltammetry	Label: AuNP nanocomposites	1–500nM	1 nM	Jiang et al. (2012)
Faradaic impedance response	Amplification: Reversible DNA nanostructure	1 nM- 2 mM	0.21 nM	Sheng et al. (2014)
Interfacial Capacitance sensing	Label free	14.5fM-14.5pM	7.8 fM	This work

4. Conclusion:

This work reports a capacitive affinity sensor based on ACEK for detection of cocaine from human serum. ACEO effect was used to speed up the transport of cocaine, which helps to develop a rapid, robust, low-cost and easy to use sensor. Without any signal amplification or use of label, the detection of cocaine molecules can be accomplished within 30 sec. As discussed earlier, the developed sensor showed a linear detection range from 14.5 fM to 1.45 pM of cocaine with a limit of detection of 7.8 fM in PBS or 1.34 pM in neat serum. The use of Proteinase K helps to break down the proteins in the serum, and as a result, no purification is needed for testing serum samples. This ACEK capacitive aptasensor has demonstrated very good sensitivity and specificity for serum-based detection, showing high potential to be implemented for on-site cocaine detection. Future study includes exploring the sensitivity and specificity in complicated biological matrices including urine.

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References

Brody, E.N., Gold, L., 2000. Aptamers as therapeutic and diagnostic agents. Rev. Mol. Biotechnol. 74, 5–13.

Castellanos, A., Ramos, A., González, A., Green, N.G., Morgan, H., 2003. Electrohydrodynamics and dielectrophoresis in microsystems: scaling laws. J. Phys. D: Appl. Phys. 36(20), 2584–2597.

Citartan, M., Gopinath, S.C., Tominaga, J., Tan, S.C., Tang, T.H., 2012. Assays for aptamer-based platforms. Biosens. Bioelectron. 34(1), 1-11.

Cheng, C., Wang, S., Wu, J., Yu, Y., Li, R., Eda, S., Chen, J., Feng, G., Lawrie, B., Hu, A., 2016. Bisphenol A Sensors on Polyimide Fabricated by Laser Direct Writing for Onsite River Water Monitoring at Attomolar Concentration. ACS Appl Mater Interfaces 8(28), 17784-17792.

Cheng, C., Cui, H., Wu, J., Eda, S., 2017a. A PCR-free point-of-care capacitive immunoassay for influenza A virus. Microchimica Acta 184(6), 1649-1657.

Cheng, C., Oueslati, R., Wu, J., Chen, J., Eda, S., 2017b. Capacitive DNA sensor for rapid and sensitive detection of whole genome human herpesvirus-1 dsDNA in serum. Electrophoresis 38(12), 1617-1623.

Cui, H., Li, S., Yuan, Q., Wadhwa, A., Eda, S., Chambers, M., Ashford, R., Jiang, H., Wu, J., 2013a. An AC electrokinetic impedance immunosensor for rapid detection of tuberculosis. Analyst 138(23), 7188-7196.

Cui, H., Cheng, C., Wu, J., Eda, S., 2013b. Rapid detection of progesterone by commercially available microelectrode chips. IEEE Sensors, pp. 1-4.

Cui, H., Wu, J., Eda, S., Chen, J., Chen, W., Zheng, L., 2015. Rapid capacitive detection of femtomolar levels of bisphenol A using an aptamer-modified disposable microelectrode array. Microchimica Acta 182(13-14), 2361-2367.

Cui, H., Cheng, C., Lin, X., Wu, J., Chen, J., Eda, S., Yuan, Q., 2016. Rapid and sensitive detection of small biomolecule by capacitive sensing and low field AC electrothermal effect. Sens. Actuators B: Chem. 226, 245–253.

Ellington, A.D., Szostak, J.W., 1990. In vitro selection of RNA molecules that bind specific ligands. Nature 346, 818.

Feng, C., Dai, S., Wang, L., 2014. Optical aptasensors for quantitative detection of small biomolecules: a review. Biosens. Bioelectron. 59, 64–74.

Hamaguchi, N., Ellington, A., Stanton, M., 2001. Aptamer beacons for the direct detection of proteins. Anal Biochem 294(2), 126-131.

Hermann, T., Patel, D.J., 2000. Adaptive Recognition by Nucleic Acid Aptamers. Science 287(5454), 820-825.

Jayasena, S.D., 1999. Aptamers: an emerging class of molecules that rival antibodies in diagnostics. Clin. Chem. 45, 1628–1650.

Jiang, B., Wang, M., Chen, Y., Xie, J., Xiang, Y., 2012. Highly sensitive electrochemical detection of cocaine on graphene/AuNP modified electrode via catalytic redox-recycling amplification. Biosens. Bioelectron. 32(1), 305-308.

Karila, L., Petit, A., Phan, O., Reynaud, M., 2010. Cocaine induced psychotic disorders: a review. Rev Med Liege 65, 632-627.

Li, Y., Ji, X., Liu, B., 2011. Chemiluminescence aptasensor for cocaine based on double-functionalized gold nanoprobes and functionalized magnetic microbeads. Anal. Bioanal. Chem. 40, 213-219.

Li, S., Yuan, Q., Morshed, B.I., Ke, C., Wu, J., Jiang, H., 2013. Dielectrophoretic responses of DNA and fluorophore in physiological solution by impedimetric characterization. Biosens Bioelectron 41, 649-655.

Li, S., Ren, Y., Cui, H., Yuan, Q., Wu, J., Eda, S., Jiang, H., 2015. Alternating current electrokinetics enhanced in situ capacitive immunoassay. Electrophoresis 36(3), 471-474.

Liu, Y., Tuleouva, N., Ramanculov, E., Revzin, A., 2010. Aptamer-Based Electrochemical Biosensor for Interferon Gamma Detection. Anal. Chem. 82, 8131–136.

Liu, X., Cheng, C., Wu, J., Eda, S., Guo, Y., 2017. A low cost and palm-size analyzer for rapid and sensitive protein detection by AC electrokinetics capacitive sensing. Biosens Bioelectron 90, 83-90.

Liu, Z., Speetjens, M.F.M., Frijns, A.J.H., van Steenhoven, A.A., 2014. Validated numerical analysis of vortical structures in 3D AC electro-osmotic flows. Microfluidics Nanofluidics 16, 1019–1032.

Maurizi, M.R., 1992. Proteases and protein degradation in Escherichia coli. Experientia 48, 178-200.

Mirzajani, H., Cheng, C., Wu, J., Ivanoff, C.S., Najafi Aghdam, E., Badri Ghavifekr, H., 2016. Design and characterization of a passive, disposable wireless AC-electroosmotic lab-on-a-film for particle and fluid manipulation. Sens. Actuators B: Chem. 235, 330-342.

Mirzajani, H., Cheng, C., Wu, J., Chen, J., Eda, S., Najafi Aghdam, E., Badri Ghavifekr, H., 2017. A highly sensitive and specific capacitive aptasensor for rapid and label-free trace analysis of Bisphenol A (BPA) in canned foods. Biosens. Bioelectron. 89, 1059-1067.

Mokhtarzadeh, A., Dolatabadi, J.E., Abnous, K., de la Guardia, M., Ramezani, M., 2015. Nanomaterial-based cocaine aptasensors. Biosens. Bioelectron. 68, 95-106.

Sheehan, P.E., Whitman, L.J., Detection Limits for Nanoscale Biosensors, 2005. Nano Letters 5, 803-807.

Sheng, Q., Liu, R., Zhang, S., Zheng, J., 2014. Ultrasensitive electrochemical cocaine biosensor based on reversible DNA nanostructure. Biosens. Bioelectron.51, 191–194.

Qiu, L., Zhou, H., Zhu, W., Qiu, L., Jiang, J., Shen, G., Yu, R., 2013. A novel label-free fluorescence aptamer-based sensor method for cocaine detection based on isothermal circular strand-displacement amplification and graphene oxide absorption. New. J. Chem. 37(12), 3998.

Wen, Y., Pei, H., Wan, Y., Su, Y., Huang, Q., Song, S., Fan, C., 2011. DNA nanostructure-decorated surfaces for enhanced aptamer-target binding and electrochemical cocaine sensors. Anal Chem 83(19), 7418-7423.

Wu, J., Ben, Y., Chang, H.-C., 2005. Particle detection by electrical impedance spectroscopy with asymmetric-polarization AC electroosmotic trapping. Microfluid. Nanofluidics 1 (2), 161–167.

Wu, J., 2008. Interactions of electrical fields with fluids: laboratory-on-a-chip applications. IET Nanobiotechnol 2(1), 14-27.

Yang, K., Wu, J., 2008. Investigation of microflow reversal by ac electrokinetics in orthogonal electrodes for micropump design. Biomicrofluidics 2(2), 24101-24108.

Yunhui, Y., 2010. Label-free electrochemical cocaine aptasensor based on a targetinducing aptamer switching conformation. Anal. Sci. 26, 1265.

Zhang, J., Wang, L., Pan, D., Song, S., Boey, F.Y., Zhang, H., Fan, C., 2008. Visual cocaine detection with gold nanoparticles and rationally engineered aptamer structures. Small 4(8), 1196-1200.

Zhang, D.W., Zhang, F.T., Cui, Y.R., Deng, Q.P., Krause, S., Zhou, Y.L., Zhang, X.X., 2012. A label-free aptasensor for the sensitive and specific detection of cocaine using supramolecular aptamer fragments/target complex by electrochemical impedance spectroscopy. Talanta 92, 65-71.