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## Applying a surface-sensitive fluorescence method to fast on-site detection of cocaine in saliva

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### Abstract

Fast and sensitive detection of cocaine in saliva is realized utilizing a surface-sensitive fluorescence measurement platform. The platform is based on a polystyrene parabolic lens that enables the simultaneous application of total internal reflection excitation (TIR) and supercritical angle fluorescence detection (SAF), which results in extreme surface sensitivity in the measurements. The molecular recognition takes place in a scheme, where cocaine molecules to be detected generate a displacement of the labelled anti-cocaine antibodies from the cocaine-BSA-conjugate molecules immobilized on a surface. The results with untreated saliva spiked with cocaine demonstrate that by monitoring the dissociation process in real time, cocaine concentrations down to 1 ng/mL can be detected within 60 s.

© 2011 Published by Elsevier Ltd. Open access under [CC BY-NC-ND license](http://creativecommons.org/licenses/by-nc-nd/3.0/).*Keywords:* Surface sensitive fluorescence; TIR; SAF; on-site detection; drugs; cocaine; oral fluids; saliva; antibody displacement.

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### 1. Introduction

Pharmaceuticals, drugs of abuse, steroids and toxins belong to a growing class of small analytes that need to be tested on different occasions by the authorities. For many drugs of abuse, oral fluids are very suitable test matrices [1], but the available commercial tests typically take several minutes to perform, which hinders their use in on-site screening [2].

In this paper, we present a potential test concept based on a recently developed fluorescence measurement platform [3, 4] and antibody displacement immunoassay. The platform has its starting point in the works of Ruckstuhl et al. [5] and Enderlein et al. [6] and it utilises simultaneously both total

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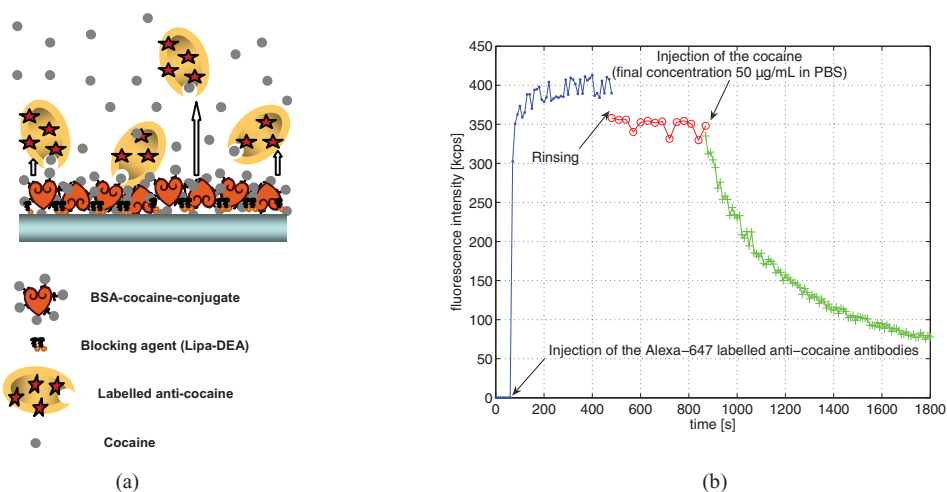


Fig. 1. (a) The principle of the cocaine-induced displacement of antibodies; (b) Fluorescence intensity recordings during the final phase of surface functionalisation, and the effect of the injection of cocaine (in PBS buffer).

internal reflection (TIR) excitation and super critical angle fluorescence (SAF) detection principles, which makes the platform extremely sensitive to surface-bound fluorescence. This provides means to monitor the displacement of the antibodies from the functionalized surface with a high sensitivity in real time.

## 2. Materials and methods

For the detection of cocaine the scheme shown in Fig. 1a was applied. The surface was first functionalized with the BSA-cocaine-conjugate and diaminomethanol derivative of lipoic acid (Lipa-DEA) serving as a blocking agent [7]. Then the labelled anti-cocaine antibody was allowed to bind to the conjugate molecules. After rinsing, the test is initiated by injecting the saliva sample. The cocaine molecules in the sample generate a displacement of antibodies from the surface, which can be observed as instant fluorescence attenuation.

Anti-cocaine antibodies and BSA-conjugated cocaine were obtained from Biosensors Applications AB. The anti-cocaine antibodies were labelled with Alexa 647 fluorescence labels (from Molecular probes) at our laboratory according to the instructions of the labelling kit.

The flat bottom microtiter wells (F96, 456537, type MaxiSorp) were purchased from NUNC. The BSA-cocaine conjugate and Lipa-DEA were diluted in PBS buffer (15 mM, PH 7.4) to concentrations of 50 µg/mL and 200 µg/mL, respectively. A volume of 50 µL was added into each well and the plate was left in dark for 15 minutes at room temperature. The wells were rinsed with PBS (5 x 200 µL), and then the anti-cocaine antibodies diluted in PBS (50 µg/mL; 50 µL/well) were left to bind in dark for 15 minutes at room temperature. After the washing step (2 x 200 µL PBS; 2 x 200 µL PBS + 0.01% Tween 20; 1 x 200 µL PBS), 50 µL of saliva was added into each well. All saliva needed in the experiments was collected during the immobilization phase and used as such without any pre-treatment. The saliva content of the final samples was over 99.5%.

The fluorescence measurements were carried out with the platform described in details elsewhere [3]. The fluorescence intensity of the functionalized surface was recorded six times a minute by counting the photons during the illumination time of 500 ms. Before the injection of the cocaine sample (diluted in 50 µL of saliva) the baseline level was recorded for 60 s.

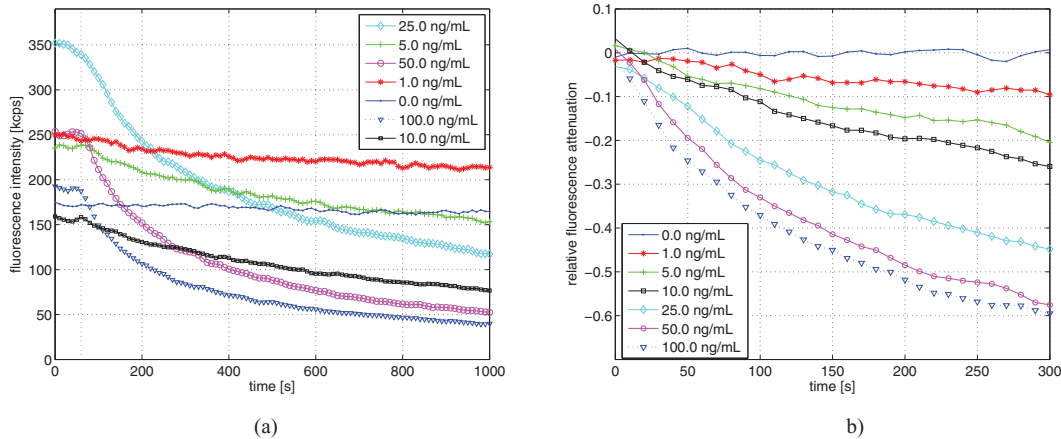


Fig. 2. (a) The fluorescence intensity recordings with different cocaine concentrations in untreated saliva; (b) The first 300 seconds of the data scaled with the baseline values and smoothed with a three point moving average.

To further illustrate the applied method, Fig. 1b shows the monitored fluorescence intensity during the final phase of the surface functionalisation and the effect of the injection of cocaine. When only PBS buffer is present in the well (coated with the BSA-conjugated cocaine and Lipa-DEA), the measured fluorescence intensity is about 0.35 kcps (kilo counts per second). Then Alexa-647 labelled anti-cocaine antibodies, diluted in 50  $\mu$ L of PBS, are injected into the well to the final concentration of 50  $\mu$ g/mL. The injection leads to rapidly increasing fluorescence intensity as the antibodies bind to the conjugate molecules at the surface. The binding process stabilizes in about eight minutes, and the well is then rinsed. After the rinsing the fluorescence signal has shifted from the level of 400 kcps to the level of 350 kcps/s, which suggests that about 15% of the antibodies were only loosely attached to the surface. During the next six minutes the signal remains stable. Then cocaine diluted in 50  $\mu$ L of PBS is injected into the well (final concentration of 50 ng/mL). This leads to a rapidly decreasing signal as the anti-cocaine antibodies are now displaced from the surface. After 15 minutes the signal level has dropped below 80 kcps/s, which suggests that almost 80% of the antibodies have been displaced.

### 3. Results and discussion

Fluorescence intensity recordings with different cocaine concentrations in saliva are shown in Fig. 2a. The response before the sample injection at the time point  $t = 60$  s serves as the baseline, which without exception is shown to be stable. After the sample injection the displacement of the antibodies is evident with all samples having cocaine, whereas no displacement can be seen with the control sample (no cocaine). It is noteworthy that the relative responses in saliva (Fig. 2a) and in PBS (Fig. 1b) are very similar. The cocaine concentration of 50 ng/mL causes a drop of 80% in fluorescence intensity in about 15 minutes both in saliva and PBS.

In Fig. 2b the relative changes during the first 300 s after the injection of the sample with respect to the baseline values are shown. The data is smoothed with a three-point moving average calculated backwards. The figure clearly illustrates that the relative changes are proportional to the cocaine concentrations, and that the concentration range of 1 – 100 ng/mL in saliva can be detected within tens of seconds.

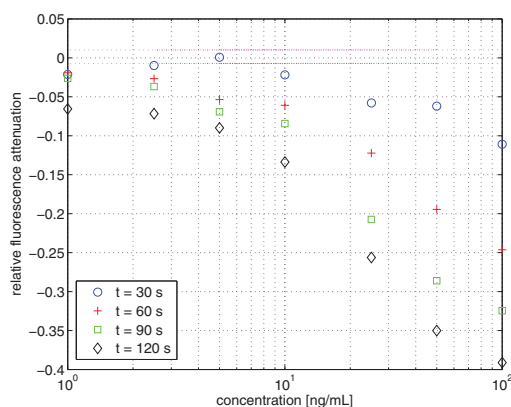


Fig. 3. The relative fluorescence attenuation at four different time points plotted against the cocaine concentration in saliva samples.

This fact is further illustrated in the Fig. 3, where the data points corresponding to the time points 30, 60, 90 and 120 seconds after the sample injection are plotted against the cocaine concentration. From this figure it can be deduced that cocaine concentrations down to 10 ng/mL or 1 ng/mL in untreated saliva can be detected in 60 s or 30 s, respectively. The current recommended oral fluid cut-offs for cocaine screening and confirmation varies between 8 and 20 ng/mL [1], so both in terms of sensitivity and speed the results are very satisfactory.

#### 4. Conclusions

A very rapid and sensitive detection of cocaine in untreated saliva was realised utilising a surface-sensitive fluorescence measurement platform together with a displacement immunoassay. The cocaine concentrations down to 1 ng/mL were reliably determined in 60 s, while the concentrations down to 10 ng/mL were shown to be detected already in 30 s. The results suggests that a device based on the surface-sensitive fluorescence reading, realised in optical plastic like polystyrene, has a high potential for initial immunoassay screening of small molecules like drugs of abuse in untreated saliva at the site of collection. Also other demanding sample matrices like serum and whole blood may be applicable with the method.

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