THE DEVELOPMENT OF A DIAGNOSTIC TEST FOR THE DETECTION OF DRUGS IN SALIVA USING A DISPOSABLE SAMPLE PREPARATION MICRO-FLUIDIC CARTRIDGE

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ABSTRACT

In this paper we report on the development of a diagnostic test for the detection of illegal drugs in saliva using a disposable micro-fluidic sample preparation cartridge which was fabricated using CO_2 and excimer laser ablation of a PMMA substrate assembly. The detection method is by immunoassay sensing on a surface plasmon resonance (SPR) platform. Experimental results shows that the immunoassay detection of the two illegal drugs tested are very sensitive and have a linear range for cocaine and MDMA of 0,01 pg/ml – 1 ng/ml and 0,1 pg/ml – 100 ng/ml respectively.

KEYWORDS: Micro-fluidic, Poly(methyl methacrylate), Saliva, Drugs

INTRODUCTION

Saliva is increasingly being used as an "ideal" sample matrix primarily as it can be collected non-invasively causing less stress to the person being tested and its composition correlates well to serum. "Point of care" (POC) diagnostic tests for illegal drugs in saliva will be becoming more important with advancing technological progress especially in "Roadside Traffic testing" where there is an immediate need for improved POC devices that can address areas concerning: specificity, sensitivity, sample collection, handling and preparation and easy non technical usability.

Oral fluid is a natural ultrafiltrate of plasma as substances are transported across epithelial membranes into oral fluid by passive diffusion across a concentration gradient. Drug transport into oral fluid is regulated by the physiochemical properties of the drug (molecular weight, dissociation constants, lipid solubility and protein binding) and the cell membrane. Based on a modified version of the Henderson-Hasselbach equation, it is generally presumed that unbound weakly basic drugs will concentrate in oral fluid, while the opposite occurs for weakly acidic drugs [1]. Low-molecular mass compounds can also be transferred into oral fluid by active secretion or diffusion through pores in the cell membrane [2]. The blood concentrations of drugs cannot easily be compared to concentrations in oral fluid but on average studies have shown a reasonable correlation [3]. Oral fluid is increasingly used as an ideal sample matrix. It can be collected non-invasively and causes less stress to the person being tested. In many countries jurisdictions have adopted the use of oral fluid to detect the presence of drugs of abuse, particularly in relation to persons driving motor vehicles. A number of studies have been made about roadside oral fluid testing[3,4,5]. Surface Plasmon resonance (SPR) is an optical phenomenon that occurs as a result of total internal reflection of monochromatic and polarized light at an interface consisting of thin gold or silver -coated prism carrying the biological component and a liquid environment, which is in direct contact with the biocomponent. The specific binding of measured analyte onto the active surface of the SPR device induces a refractive index change that can be monitored. The advantage of this method over most other optical sensors is that loading of the surface with a receptor element as well as interactions with analytes fitting to the receptor can be monitored in real time without any additional labeling. SPR technology is dependent on fluid being moved across the sensing surface and also is highly sensitive to any substance that could bind to the gold sensing surface. Therefore any inferring substances that could non-specifically bind to the sensing surface of which there are many in saliva (e.g. mucin) would need to be removed. This is where micro-fluidics can provided a solution.

THEORY

The aim of this study was to develop a diagnostic test for the detection of illegal drugs in saliva [6]. Here we describe a sample preparation and analytical detection method that addresses many of these problems outlined above. A disposable micro-fluidic system was developed where a saliva sample could be directly injected into the unit as shown in figure 1 and 2. If there were any drugs in the sample, the small drug molecules can travel through the partition into the carrier analysis buffer, which will be afterwards transported directly to the sensor surface. In this way, large interfering molecules presents in saliva are filtered out and difficult sample treatment procedures are avoided.

EXPERIMENTAL

The cartridge is micro-fabricated out of PMMA , medical grade pressure sensitive acrylic tape and a sample/buffer partition. The micro-fluidic channels were formed by using CO_2 and excemier laser ablation. In the bottom of the buffer channel a staggered herringbone mixer (SHM) has been fabricated. The herringbone mixer consists of grooves in the bottom of the channel placed at an angle of 45º to the flow direction. These grooves create vortices in the flow and by placing them in a staggered herringbone formation an efficient mixer is created as previously described by Strooks *et al* [7]. The sensor platform is based on surface plasmon resonance (SPR) that uses a 670 nm laser diode, [SPR Navi 200] (figure 1). The fabricated sample preparation cartridge was easily integrated onto the SPR equipment (figure 2). An inhibition immunoassay is used as the detection method. A protein- analyte conjugate i.e. Ovalbumin-MDMA (OVA-MDMA) and Ovalbumin- Cocaine (OVA-Coc) where physically adsorbed on to different channels (50µg/ml and

100µg/ml respectively) overnight to surface modify the gold sensing surface to be able to capture the detecting related anti-bodies against the drug analytes. The monoclonal antibodies that specifically recognise and capture illegal drugs are injected into the analysis buffer (50µg/ml). Therefore, if a drug has been carried into the carrier buffer, reduced binding will be observed on the corresponding sensing surface.

Figure 1: SPR Navi 200

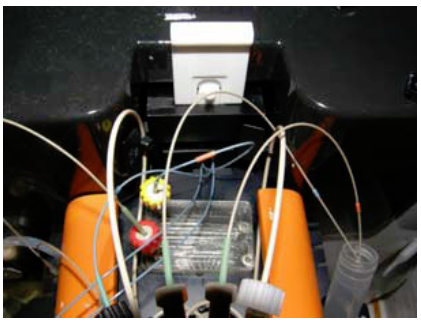

Figure 2: Integration of sample preparation cartridge on to a SPR Navi 200 device

RESULTS AND DISCUSSION

Fabrication of the sample preparation cartridge was fairly straight forward to fabricate. It was noted that the units required heat pressing for ~20min when after assembled to press out any trapped air bubbles in the adhesive layer. The assembled cartridge can be seen in figure 3. The cartridge was fabricated with different sample inlet diameters to allow different sample injection methods i.e, pipette, syringe and directly form a saliva collection device fitted with a 5ml syringe plunger (see figure 4).

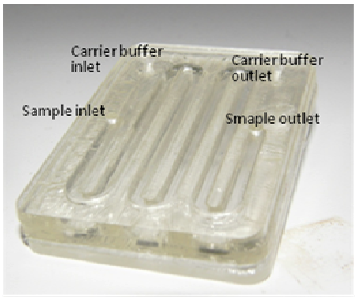

Figure 3. Sample preparation cartridge

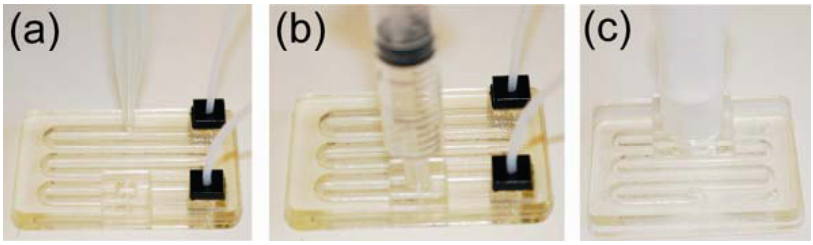

Figure 4. Three different sample injection methods. (a) using a pipette, (b) using a syringe, and (c) using a saliva sample pad device "*Salivette*, Sarsted"

The extraction efficiency of the sample preparation cartridge was evaluated using the developed immunoassay on a micro titre plate sing 10 ng/ml drug spiked samples. The results obtained displayed higher extraction for spiked saliva samples using the fabricated cartridge compared to using the saliva sample directly which showed a decreased in signal due to non-specific binding (see figure 5). As it is not possible to get 100% extraction of analyte into the carrier buffer, at 80-90% extraction a sample containing 1ng/ml will still be able to be determined.

As SPR is a highly sensitive, fast, label free and real time analytical technique for the detection of molecular interactions, the analysis detection time currently takes ~7 minutes. Experimental results shows that the immunoassay detection of the two illegal drugs tested are very sensitive and have a linear range for cocaine and MDMA of 0,01 pg/ml – 1 ng/ml and 0,1 pg/ml – 100 ng/ml respectively . As the immunoassay is very sensitive saliva samples containing 5ng/ml using the sample preparation cartridge can be detected (see figure 6). These results are comparable if not better then rapid drug test commercially available. Where typically, detection cutoffs for MDMA and cocaine are 25ng/ml and 12ng/ml respectively, detected in ~10-13mins

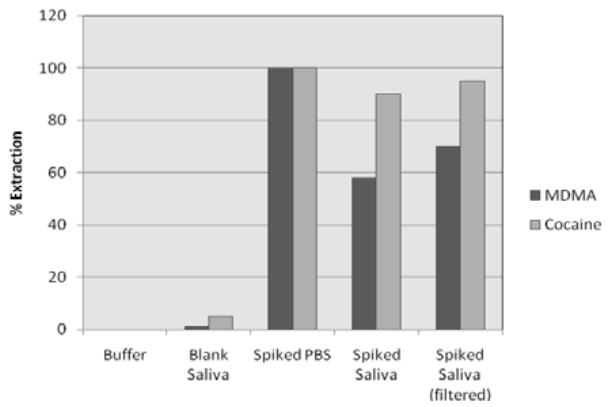

Figure 5: Sample extraction efficiency of the micro-fluidic cartridge for two different drugs (i.e. cartridge result=filtered)

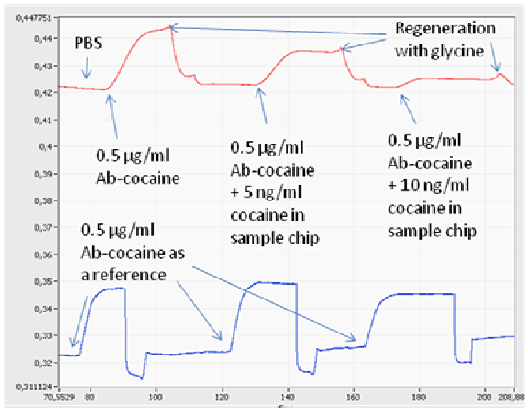

Figure 6: SPR sensorgram showing the sensitivity of cocaine detection using the sample preparation cartridge.

CONCLUSION

In the study we introduce a novel micro-fluidic sample preparation cartridge that can be integrated on to a SPR platform for the detection of illegal drugs in saliva samples. Our data shows that saliva samples containing of 5ng/ml of cocaine and MDMA can be detected within 7 minutes. With further optimization of the assay we are aiming to reduce our detection time to 3 minutes.

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