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Design and Synthesis of a fluorescent Molecular Imprinted Polymer for use in an Optical Fibre-Based Cocaine Sensor

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ABSTRACT

Previously, we have developed chemical sensors using fibre optic-based techniques for the detection of Cocaine, utilising molecularly imprinted polymers (MIPs) containing fluorescein moieties as the signalling groups. Here, we report the computational design of a fluorophore which was incorporated into a MIP for the generation of a novel sensor that offers improved sensitivity for Cocaine with a detection range of 1-100μM. High selectivity for Cocaine over a suite of known Cocaine interferants (25μM) was also demonstrated by measuring changes in the intensity of fluorescence signals received from the sensor.

Keywords: Optical fibre sensor, Cocaine sensor, chemical sensor, molecular imprinting, computational design.

1. INTRODUCTION

The detection of Cocaine has been extensively investigated[1,2] and highly sensitive detection of this substance is vital for law enforcement and health-related reasons. Development of more reliable and more cost-effective methods of drug detection are important[3] since existing technologies are beset by problems such as high cost, low portability, low sensitivity and false alarms. Sensing solutions for Cocaine detection have been reported, e.g. aptamer-based biosensors[4-7] and sensors for saliva,[8] but development of a compact, hand-held monitor utilising stable synthetic molecular receptors remains of interest for some specific applications. Optical fibre sensors can provide many advantages over other sensing technologies and these include their lightweight nature, low cost, potential for multiplexing, remote sensing capability, immunity to electromagnetic interference and overall robustness. Herein, we describe a novel Cocaine sensor based on an optimised version of our earlier probes that combines optical fibre sensing with the use of a molecularly imprinted polymer (MIP).[3,9] MIPs are durable, synthetic molecular receptors with enhanced stability compared to biological receptors. We aimed to identify a sensor that allows rapid real time drug detection with the capability of distinguishing between Cocaine and legal impurities (interferants). The optical fibre itself facilitated the guidance of excitation light to the sensor material and the collection of the fluorescence signal generated when the sensor material interacts with Cocaine. In our approach (Figure 1) a complex is formed between the carboxyl group on a polymerisable fluorophore and the amine group present in Cocaine (analyte). The complex is copolymerised with cross-linking monomer on the end surface of the fibre, which itself has been functionalised with polymerisable groups. After extraction of the polymer the resultant MIP formed on the end surface of the fibre contains recognition sites incorporating the fluorophore and thus exhibits an increase of fluorescence intensity selectively in the presence of the analyte.

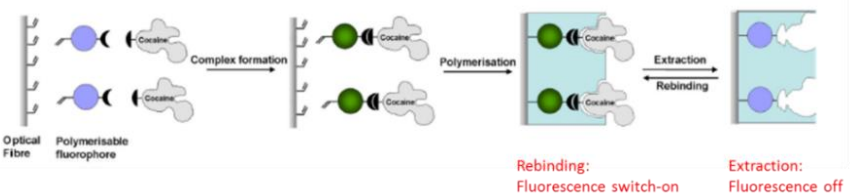

Figure 1. Fluorescent MIP sensor approach for Cocaine sensing

2. COMPUTATIONAL CHEMISTRY

Our strategy for the design of an improved fluorescein-based Cocaine sensor involved a modelling approach. The use of computational design to optimise the composition of MIPs has been previously described.[10] Briefly, the chemical structure of Cocaine was drawn and its energy minimised in acetonitrile, the solvent of choice in the polymerisation step, and screened against a virtual library containing 13 monomers using the LEAPFROG^{TM} algorithm. Typically 30,000 iterations through possible binding sites of the monomers with the target were needed to exploit all possible site interactions. The monomers giving the strongest binding interactions with Cocaine were identified. After taking into consideration chemical tractability, MNEW (fluorophore **1**) with a binding energy, of -25.42 kcal mol^{-1}, was used as the preferred candidate for our new sensor.

3. ORGANIC SYNTHESIS

Synthesis of **1** was acheived in 4 steps from 2-methoxy4-nitrobenzoic acid, as outlined in Figure 2. This fluorophore was then used in the sensor fabrication.

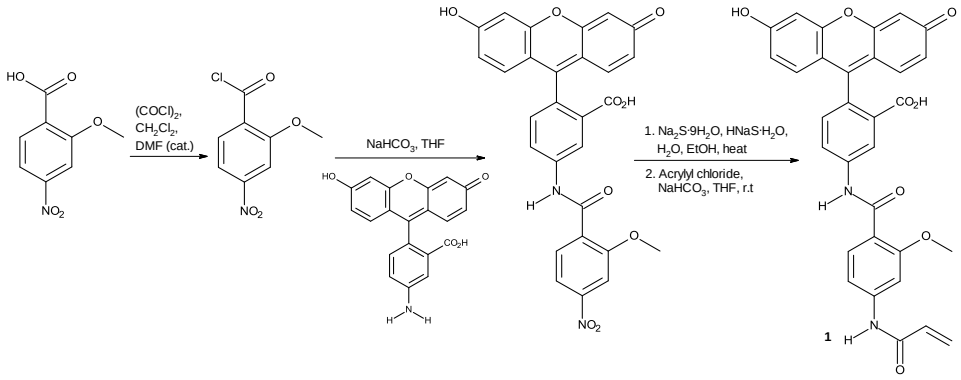

Figure 2. Synthesis of **1** (MNEW)

4. SENSOR PROFILING

4.1 Sensor Fabrication and Experimental Set-Up

The fabrication of the Cocaine sensing probe requires a multi-step process.[3]The distal end of a 1000μm diameter UV multimode fibre purchased from Thorlabs was polished, treated (10% KOH in isopropanol then Piranha solution), then modified by silanisation in a 10% solution of 3-(trimethoxysilyl) propyl methacrylate in dry ethanol. The fibre was then washed with ethanol repeatedly in an ultrasonic bath. The pre-polymerization mixture was prepared by dissolving Cocaine (2 equivalents), fluorophore **1** (1 equivalent), ethylene glycol dimethacrylate cross linker (80 equivalents), methacrylic acid co-monomer (14 equivalents) and AIBN initiator (0.7 mg) in dry acetonitrile. This solution was left standing for 2 hrs then purged thoroughly with argon for 5 min and a small volume of the mixture was placed into a capillary tube and the distal end of the fibre was inserted. This arrangement was sealed quickly with melted plastic and polymerised occurred at 70^{o}C. A MIP layer formed on both the cylindrical surface and the distal end surface of the fibre. However, only the MIP on the distal end surface is responsible for sensing as only this part of the sensor material is excited by light transmitted by the fibre. The sensor tip was washed repeatedly for 8 hrs (methanol:acetic acid (4:1, v/v) followed by methanol alone) using intermittent ultrasonication. A control probe (non-imprinted polymer, NIP) was prepared, under identical conditions, using the same protocol but without the addition of Cocaine.

The set-up used for the measurements undertaken to calibrate the probe is described as follows.[3] Light from a 375 nm LED was coupled through a multimode UV/Visible fibre with hard polymer cladding, 1000μm silica core and numerical aperture (NA) of 0.37, using collimation and focusing lenses, into a 2x1 Y fibre coupler. The far end of the coupler, made using two multimode UV/Visible fibres with hard polymer cladding, 600μm silica core and 0.37 NA, was connected to the sensor probe with the active sensing region being located at the distal end of the fibre. Following

interaction of Cocaine with the active region, a portion of the total light emitted from the sensing layer was collected and guided through the same fibre bundle to the other branch of the fibre coupler which is connected to an Ocean Optics USB2000 spectrometer, the output from which was then displayed on a computer screen (using SpectraSuite software).

4.2. Assessment of Sensor Performance

4.2.1 Sensitivity to Cocaine

The calibration measurements of the sensor were performed using the preferred distilled water:acetonitrile (9:1) solvent system[9] over 15 minutes and a methanol rinse was performed after each measurement (progressing from low to high analyte concentrations). A 1000μM stock solution of Cocaine was diluted appropriately to provide the test solutions; 1 in 40 (25μM), 1 in 20 (50μM), 1 in 10 (100μM), 1 in 4 (250μM). The sensor exhibited good sensitivity and responded between 25μM and 100μM Cocaine response (Figure 3a) and it appeared to be saturated using 250μM Cocaine; short-term washing gave a fluorescence response below background peak (when zero Cocaine present). The response of the control/NIP probe to Cocaine was also studied and no increase in fluorescence (rather a 1.4% decrease) was observed upon addition of 25μM Cocaine (at 523.6nM) relative to when zero cocaine was present). Conversely, the MIP probe exhibited a 6.1% increase in fluorescence at this wavelength. These results suggest that the analyte bound to the MIP more strongly than to the NIP and confirming the existence of effective MIP recognition. After this initial study, the probe was washed for a further ~8 hrs with methanol:acetic acid (4:1) followed by methanol, with periodic insertion into an ultrasonic bath, before generating data shown in Figure 3b. Here, the sensor became saturated (500μM measurement gave lower signal) so once again appeared to have a maximum detection limit. Although the background (no Cocaine) peak was 'lowered' the sensor regenerated and still functioned (see 25μM trace). Pleasingly, the lowest concentration of Cocaine that caused a distinguishable change in fluorescence intensity was 1μM and this low detection limit provided an advantage over previous sensors[3,9] of this type (2-500μM).

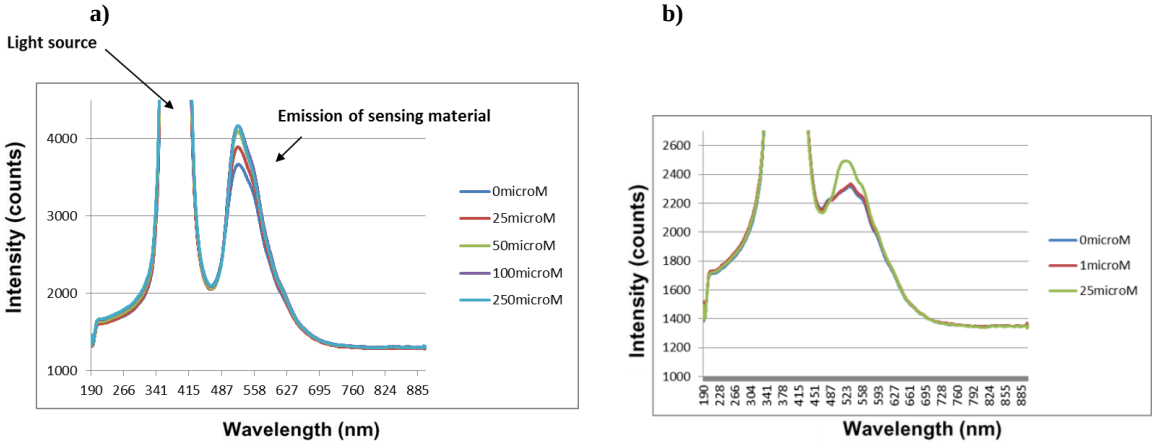

Figure 3: Measurement results (all in H_2O:MeCN 9:1). **a)** Sensor probe's response to Cocaine (after 8 hr pre-wash). **b)** Response of sensor probe to low Cocaine concentrations (after second 8 hr wash).

4.2.2. Selectivity (interferent testing)

Cocaine is often combined with a variety of other 'interferants' so further tests were undertaken to assess the sensor's response to selected masking agents. Interferant solutions were prepared using concentrations of 0.30 mg/mL (in 9:1 water:acetonitrile) which were then diluted appropriately to afford testing solutions equivalent to 25μM Cocaine in weight. The sensor was first washed for 4 hrs (4:1 methanol:acetic acid) then 3 hrs (methanol). The sensor was allowed to equilibrate for 10 mins for each measurement and was rinsed with methanol after each measurement. The sensor showed good selectivity by responding less to all the interferants shown in Figure 4a-c compared to Cocaine (at 25μM; wavelength = 523.6).

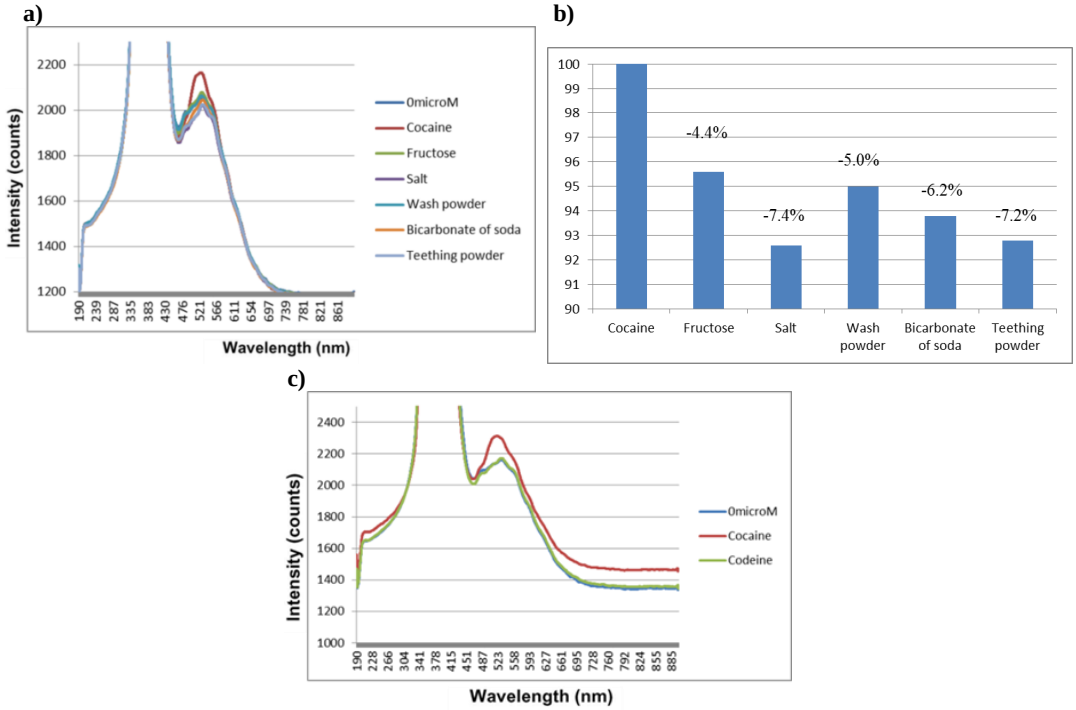

Figure 4: Measurement results (all in H_2O:MeCN 9:1). **a)** Sensor probe's response to Cocaine and known masking agents (at 25μM). **b)** Alternative depiction of interferant testing (fluorescence intensity relative to Cocaine). **c)** Sensor probe's response to Codeine compared to Cocaine (at 25μM)

5. CONCLUSIONS & FUTURE OUTLOOK

Rational sensor design studies have allowed the engineering of a sensor – derived from **1** - that displays enhanced sensitivity for Cocaine over earlier generation probes[3,9] and encouraging selectivity for Cocaine over other agents. Testing this new sensor against a wider range of interferants is now underway. In addition, further profiling and refinement of other drug sensor designs is in progress and their performance will be compared in terms of repeatability and photostability before devices can be tested in the field.

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