Preparation of a Novel Drug Sensor using a Molecular Imprinted Polymer Approach

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ABSTRACT

A chemical sensor for the detection of cocaine has been developed, based on a molecularly imprinted polymer (MIP) containing a fluorescein moiety as the signalling group. The fluorescent MIP was formed and covalently attached to the distal end of an optical fibre. The sensor exhibited an increase in fluorescence intensity in response to cocaine in an aqueous acetonitrile mixture. Selectivity for cocaine over codeine has been demonstrated.

Keywords: Optical fibre sensor, chemical sensor, cocaine sensor, fluorescence, molecular imprinting.

INTRODUCTION

The detection of cocaine has been extensively investigated due to the adverse health effects and related dangers associated with its illicit use.[1,2] Sensitive detection of cocaine is critically important for law enforcement and clinical diagnostics. Existing technologies for drug detection include manual handling, but it is easy to miss concealed items and its operation is time-consuming. Employment of sniffer dogs involves a high cost and is complicated by limited duty cycles and false alarms. Other competing technologies include Raman Spectrometry (uses delicate lasers and expensive), Ion Mobility Spectrometry (has been widely used for multi-drug screening, low sensitivity and selectivity, gives false alarms), Fourier Transform Infrared Spectroscopy (high false alarm rate), Gas Chromatography Mass Spectrometry[3] and Liquid Chromatography Mass Spectrometry (samples would require testing at remote sites, systems are bulky and expensive). In addition, field ID Kits (e.g. drug wipes) make use of expensive consumables and delicate equipment. Also, biosensors[4,5] are fragile and costly. Floor standing systems are non-portable and operate over short ranges. Their disadvantages include poor effectiveness when moisture is present in air; low specificity (Terahertz); high cost and safety concerns (X-ray, Z Backscatter scanning).

Optical fibre sensors can, however, offer many advantages over the above sensing technologies highlighted and these include small size and lightweight with low or competitive cost, showing the potential of multiplexing multiple sensors on a single fibre network and of remote sensing capability. The sensors are in particular suitable for working in harsh environments due to their immunity to electromagnetic interference, chemically resistance. In addition, utilising the molecularly imprinting polymer (MIP) technique is a key strength because preparation of synthetic molecular receptors allows recognition of any given target molecule. Other advantages are robustness, thermal and chemical stability, low cost and long shelf-life and MIP-based sensing provides a more stable alternative to biological receptors.

2. SENSOR DESIGN STRATEGY

This research has aimed to develop a stable, compact and portable sensing system capable of real time drug detection and builds on our previous work[6,7] by further optimisation of a fluorescein-based scaffold interacting with cocaine. A combination of molecular imprinting (as a method for generating chemically selective binding sites) and fluorescence modulation (as a means of signalling the presence and concentration of the analyte) was used in the sensor material design.

The MIP receptor which is selective for cocaine was covalently bonded to the distal end surface of an optical fibre, as illustrated in Figure 1. The optical fibre itself facilitated the guidance of excitation light to the sensor material and collection of the fluorescence signal generated when the sensor material interacts with the target molecules (cocaine).

The imprinting and sensing approach is also illustrated in Figure 1. A complex is formed between the carboxyl group on the 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 has been functionalised with polymerisable groups. Then the analyte is extracted from the polymer. The resulting 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.

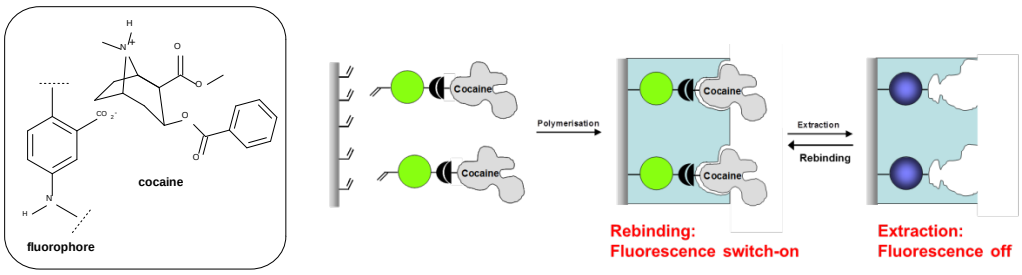

Figure 1: Fluorescent MIP sensor for cocaine

The strategy employed to enhance sensor selectivity for cocaine was two-pronged. Firstly, it was hoped that additional interactions between the fluorophore and the drug could be found by subtle monomer design; however we recognised that this may not be facile considering the distance between the amine group and the two ester groups of cocaine. A second option involved targeting monomers that bear different functional groups to acrylamidofluorescein.[6]

3. EXPERIMENTAL

3.1 General

All chemicals were of analytical grade, purchased from Sigma-Aldrich or Acros Organics and were used without further purification except for ethylene glycol dimethacrylate which was distilled under reduced pressure. The novel fluorophore was prepared from 5-aminofluorescein according to standard procedures. The solvents used for synthesis were either of HPLC grade (from Fisher Scientific) or anhydrous (from Sigma-Aldrich). Dry ethanol and dry acetonitrile for probe fabrication were taken from sealed bottles under argon. All aqueous solutions were prepared using distilled water.

3.2 Sensor probe fabrication

The fabrication of the cocaine sensing probe (Figure 2) requires a multi-step process.[6] The distal end of a 1000 m diameter UV multimode fibre purchased from Thorlabs was polished and washed with acetone. The distal end was then immersed in 10% KOH in isopropanol for 30 min with subsequent rinsing in copious amounts of distilled water and dried with compressed nitrogen. Subsequent treatment with a 30:70 (v/v) mixture of (30%) and (conc.) (Piranha solution), for 30 min, was followed by rinsing in distilled water for 15 min and drying at 100^{o}C for 30 min. This procedure left the surface with exposed hydroxyl groups which facilitated bonding of a silane agent. The fibre surface was then modified by silanizing for 2 h 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 equivalent), ethylene glycol dimethacrylate cross linker (80 equivalents), acrylamide co-monomer (14 equivalents) and 2,2'-azobisisobutyronitrile initiator (1.1 mg) in dry acetonitrile. The solution was purged thoroughly with argon for 10 min. A small volume of the solution was placed into a capillary tube via syringe and the distal end of the fibre was inserted. They were sealed quickly with PTFE tape and polymerized in an oven at 70^{o}C. This procedure formed a MIP layer 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 therefore its fluorescence signal generated is collected by the fibre before being guided to a mini-spectrometer for spectral analysis. The sensor tip was washed repeatedly with MeOH-AcOH (8:2, v/v) in an ultrasonic bath, followed by the same procedure with MeOH alone to remove the cocaine and all unreacted materials and the excess amount of polymer formed which was not directly bound to the fibre. Also, the probe was washed in this way (to remove bound analyte) after each

measurement. A control probe (non-imprinted polymer, NIP) was prepared at the same time under identical conditions, using the same protocol but without the addition of the cocaine.

3.3 Experimental set-up

The set-up used for the measurements undertaken to calibrate the probe is shown in Figure 2, where light from a LED, emitting at a centre wavelength of 375 nm, 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).

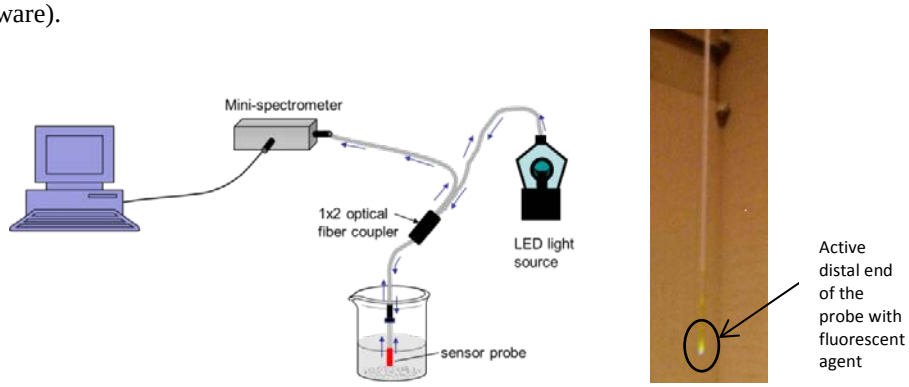

Figure 2: a) Sensor system & new cocaine probe b) showing the active distal end

4. RESULTS AND DISCUSSION

4.1 Response time of the sensor

Before performing calibration measurements of the sensor, its response time was investigated (in H_2O:MeCN 9:1). It took approximately 15 minutes for the sensor to attain equilibrium and this result compares favourably with other MIP sensor systems.[8,9]

4.2 Response of the sensor to cocaine

The calibration measurements of the sensor were performed using the preferred H_2O:MeCN 9:1 solvent system[6] over 15 minutes. The sensor responded to 1000µM cocaine and exhibited minimal response at lower concentrations (Figure 3). The response of the control/NIP (non-imprinted polymer) probe to cocaine was also studied and no increase in fluorescence (rather a 1.19% decrease) was observed upon addition of 1000µM cocaine (at 526.08 nM) relative to when zero cocaine was present, Figure 4b). Conversely, the MIP probe exhibited a 16% increase in fluorescence. These results suggest that the analyte bound to the MIP more strongly than to the NIP and confirming the existence of effective MIP recognition.

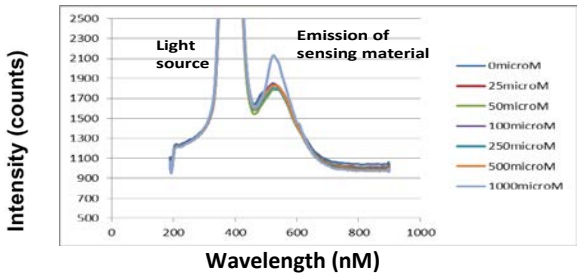

Figure 3: Fluorescence spectra of the sensor

4.3 Response of the sensor to different drugs

Investigation of the probe's selectivity to cocaine compared to other drugs was deemed important because it is often combined with other materials. Previously,[6] significantly higher reactivity of the sensor for codeine was observed compared to that for other agents. A positive indication is seen in Figure 4a which shows that the sensor responds far less to codeine than cocaine (1000μM, in 9:1 H_2O:MeCN; 3% fluorescence enhancement compared to 16%, at 526.08 nM).

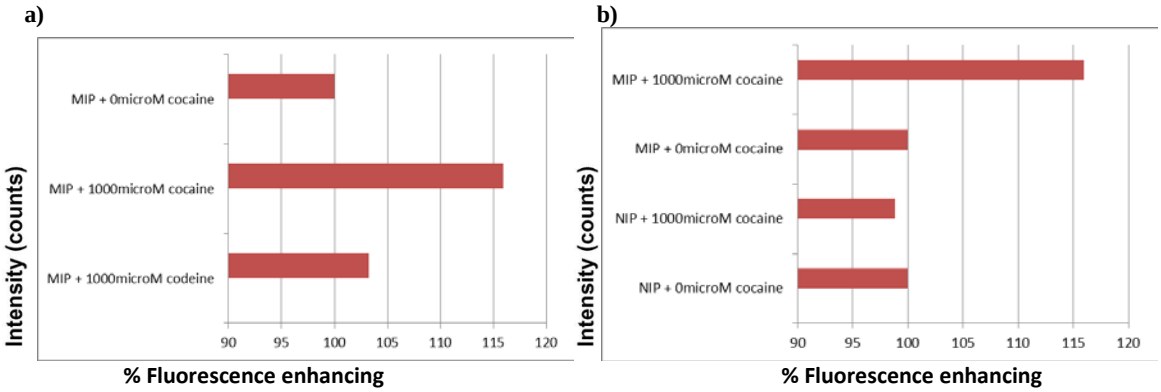

Figure 4: Measurement results (all in H_2O:MeCN 9:1).
a) Sensor probe's response to codeine and cocaine (concentration of 500μM).
b) Response of control & sensor probes to 1000μM cocaine

5. CONCLUSIONS

In this paper, further enhancement of a chemical sensor for cocaine is reported. The sensor showed an increase of fluorescence intensity in response to cocaine at 1000μM in an aqueous acetonitrile mixture. Better selectivity for cocaine over codeine was also demonstrated. Studies are underway to further optimise the performance of sensors of this type by tuning both the sensor material design and conjugated fluorophores for signalling purposes. This work has significant potential for commercial applications in the homeland security field.

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