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Intrinsic Fluorescence-Based Optical Fiber Sensor for Cocaine Using a Molecularly Imprinted Polymer as the Recognition Element

T. Hien Nguyen, Sheila A. Hardwick, Tong Sun, and Kenneth T. V. Grattan

Abstract—A fiber-optic 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 fiber. The sensor exhibited an increase in fluorescence intensity in response to cocaine in the concentration range of 0 – 500 μM in aqueous acetonitrile mixtures with good reproducibility over one month. Selectivity for cocaine over others drugs has also been demonstrated.

Index Terms—Cocaine sensor, fluorescein, fluorescent sensor, molecular imprinting, optical fiber sensor.

I. INTRODUCTION

COCAINE is one of the most commonly abused drugs and this has led to extensive investigative research efforts for its detection, due to the adverse health effects and related dangers associated with its illicit use [1], [2]. There are several major analytical methods available for the analysis of cocaine and its metabolites including gas chromatography mass spectrometry (GC/MS) [3], [4], high performance liquid chromatography (HPLC) [5], [6], thin layer chromatography [7], voltammetry [8], radioimmunoassay [9] and enzyme-linked immunosorbent assay (ELISA) [10]. These traditional methods, despite having achieved very good results, are generally expensive, time consuming and cumbersome for real-time measurements outside the laboratory, some of which also require sample clean-up and derivatization of cocaine prior to analysis. Biosensors, which rely on the specificities of the binding sites of receptors, enzymes, antibodies or DNA as biological sensing

elements, have been considered as alternative analytical devices due to their specificity, portability, speed and low cost [11]. Biosensors for cocaine based on monoclonal antibodies [12], [13] and especially aptamers [14]–[18] have been developed in recent years. However, these sensors suffer from certain limitations in light of their potential practical applications in the field due to the fragile and unstable nature of the biological recognition elements. Therefore, the development of stable, compact and portable sensing systems which are capable of real time detection of the target drug remains a compelling goal which is addressed in this work.

Molecular imprinting has been extensively demonstrated over the last three decades as a versatile technique for the preparation of synthetic molecular receptors capable of the selective recognition of given target molecules. The approach is based on the self-assembly of a template molecule with polymerizable monomers possessing functional group(s) interacting with the template [19], [20]. After polymerization, the template is removed, leaving vacant recognition sites which are complementary in shape and functional groups to the original template. Molecularly imprinted polymers (MIPs) provide an exciting alternative to biological receptors as recognition elements in chemical sensors [21]. In this research, a robust fiber optic chemical sensor for cocaine detection has been developed, based on the combination of molecular imprinting (as a method for generating chemically selective binding sites) and fluorescence modulation (as a means of signaling the presence and concentration of the analyte). The attraction of this approach lies in the advantages offered both by the optical fiber in terms of small size, immunity to electromagnetic interference, remote sensing capability, resistance to chemicals and biocompatibility [22], [23] and by the synthetic polymer receptor in terms of robustness, thermal and chemical stabilities, low cost and long shelf-life [20]. The molecularly imprinted polymer (MIP) receptor which is selective for cocaine was covalently bonded to the distal end of the optical fiber, which facilitated rapid and highly sensitive detection. Acrylamidofluorescein (AAF) was used as fluorescent functional monomer interacting with the template cocaine. The sensing mechanism depends on changes in the frontier orbitals of fluorescein, which occur when it is deprotonated by a base. The deprotonated form is fluorescent and the protonated form is much less so. In the presence of cocaine, the carboxylate group of AAF is deprotonated. Cocaine acts as a base in the ion pair complex, accepting a proton from AAF and leading to an increase in the observed fluorescence intensity (Fig. 9). The imprinting and sensing

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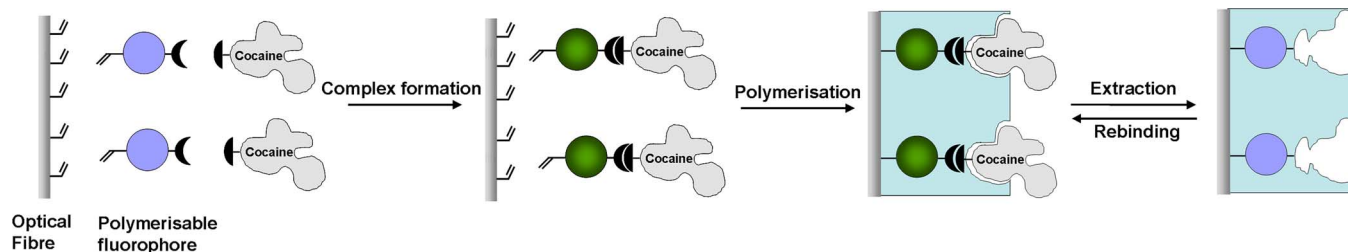


Fig. 1. Preparation of a cocaine sensing MIP on the surface of the optical fiber which exhibits fluorescence changes upon template binding.

strategy is illustrated in Fig. 1. A complex is formed between the functional group -COOH on the fluorophore and the amine group on the template/analyte. The complex is co-polymerized with a cross-linking monomer and co-monomer on the surface of the fiber, which has been functionalized with polymerizable groups. Then the template/analyte is extracted from the polymer. The resulting MIP formed on the fiber contains recognition sites incorporating the fluorophore and exhibits an increase in fluorescence intensity selectively in the presence of the template/analyte. As a result, the selectivity of the sensor has been designed to arise from the functional group of the fluorophore and from the shape of the cavity.

II. EXPERIMENTAL APPROACH

A. Sensor Probe Fabrication

All chemicals were of analytical grade, purchased from Sigma-Aldrich and were used without further purification, except for ethylene glycol dimethacrylate (EDMA) which was distilled under reduced pressure prior to use. AAF was prepared from fluoresceinamine according to the literature procedure [24] as shown in Fig. 10. All solvents used were of HPLC grade from Fisher Scientific. Dry ethanol and dry acetonitrile for probe fabrication were taken from sealed bottles under argon. All aqueous solutions were prepared using distilled water. Absorption and fluorescence measurements of aqueous solutions containing fluorophore were carried out on a PerkinElmer Lambda 35 spectrophotometer and a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer system with FluorEssence™ as driving software, respectively.

The fabrication of the cocaine sensing probe requires a multi-step process which is described below. The distal end of a 1000 μm diameter UV multimode fiber purchased from Thorlabs was polished in succession with 5 μm , 3 μm and 1 μm polishing pads (Thorlabs) 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. After that, it was treated in a 30:70 (v/v) mixture of H_2O_2 (30%) and H_2SO_4 (conc.) (Piranha solution) for 30 min, rinsed in distilled water for 15 min and dried in an oven at 100°C for 30 min. This procedure leaves the surface with exposed hydroxyl groups which facilitate bonding of a silane agent. The fiber surface was then modified by silanizing for 2 h in a 10% solution of 3-(trimethoxysilyl) propyl methacrylate in dry ethanol. The fiber was washed with ethanol repeatedly in an ultrasonic bath. Subsequently, it was dried in an oven at 70°C for 2 h. This

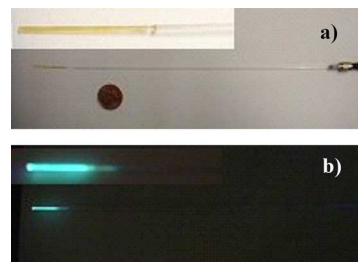


Fig. 2. Cocaine probe prepared in this work showing the active distal end of the sensor a) under normal conditions and b) when 375 nm ultraviolet (UV) light was launched to the end of the fiber.

procedure functionalizes the fiber surface with polymerizable acrylate groups.

The pre-polymerization mixture was prepared by dissolving cocaine (6.1 mg, 0.02 mmol), AAF (4.0 mg, 0.01 mmol), ethylene glycol dimethacrylate cross linker (150.9 μL , 0.8 mmol), acrylamide co-monomer (10.0 mg, 0.14 mmol) and 2,2'-azobisisobutyronitrile initiator (1.1 mg) in 222 μL dry MeCN. 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 fiber was inserted. They were sealed quickly with PTFE tape and polymerized in an oven at 70°C for 16 h. This procedure forms a MIP layer on both the cylindrical surface and the distal end surface of the fiber. However, only the MIP on the distal end surface is responsible for the fluorescence signal which is produced by direct excitation from the light source. The MIP on the side plays no role in the sensing process since evanescent wave excitation is eliminated by keeping the cladding of the fiber intact. The probe prepared by this procedure is shown in Fig. 2 where it can be seen that the distal end of the probe shows a distinctive coloration due to the presence of the fluorophore. 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 template and all unreacted materials and the excess amount of polymer formed which was not directly bound to the fiber. The probe was then stored in a cool and dark place until use. A control probe (non-imprinted polymer, NIP) was prepared at the same time under identical conditions, using the same recipe but without the addition of the template cocaine.

B. Experimental setup Used for Measurement

The setup used for the measurements undertaken to calibrate the probe is as presented in Fig. 3, where light from a LED, emitting at a centre wavelength of 375 nm is coupled through a mul-

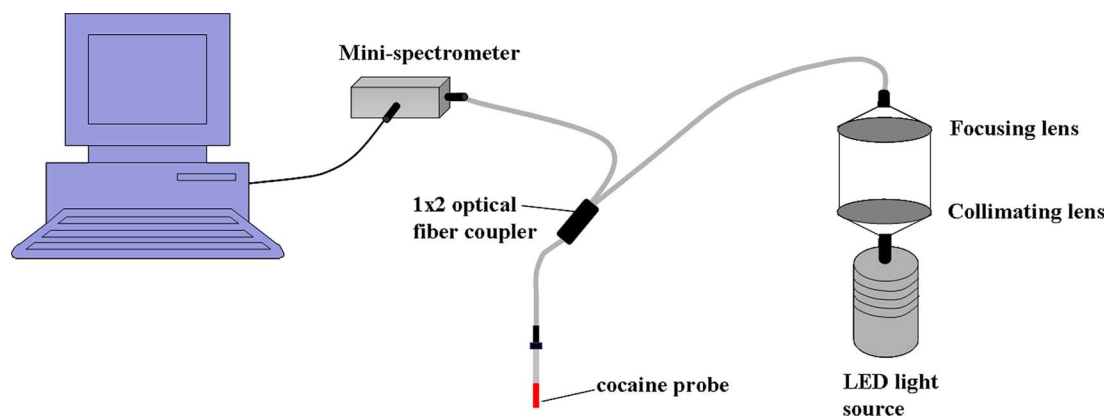


Fig. 3. Experimental setup used in the evaluation of the performance of the probe designed.

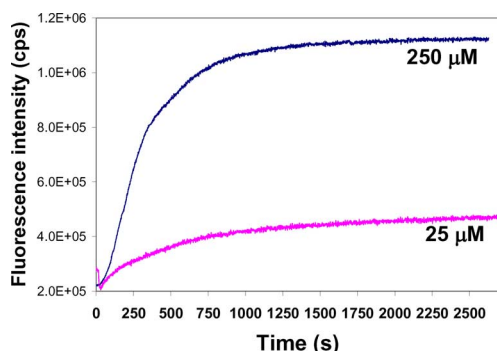


Fig. 4. Dynamic response of the sensor probe at 515 nm (excitation at 375 nm) showing the 15 min response time (to 95%).

timode UV/Visible fiber with hard polymer cladding, 1000 μm silica core and numerical aperture (NA) of 0.37, using collimation and focusing lenses, into a 2×1 Y fiber coupler, made using two multimode UV/Visible fibers with hard polymer cladding, 600 μm silica core and 0.37 NA, which is connected to the sensor probe with the active sensing region being located at the distal end of the fiber. Following interaction of cocaine with the active region, a portion of the total light emitted from the sensing layer is collected and guided through the other end of the fiber coupler to an Ocean Optics USB2000 spectrometer, the output from which is then displayed on a computer screen.

III. RESULTS AND DISCUSSION

A. Response Time of the Sensor

Before performing measurements to calibrate the sensor, its response time was investigated. Fig. 4 shows the dynamic response of the sensor obtained from the spectrofluorometer to a step change from no cocaine present (0 μM) to 25 μM and to 250 μM cocaine in $\text{H}_2\text{O}/\text{MeCN}$ 9:1. Although around 70% of the total signal change occurred within five minutes, it took around fifteen minutes for the sensor to attain equilibrium (to 95%) in 250 μM cocaine and twenty minutes in 25 μM cocaine. The higher concentration of cocaine appeared to give a slightly quicker response time. However, the difference was not significant. This response time is considered to be rapid compared to

other MIP sensor systems where a few hours incubation is required for the interaction between the template/analyte and the binding sites in the MIP to reach equilibrium [25], [26]. This important result is most probably due to both the intrinsic sensor design and the thickness of the polymer film since the thicker the polymer layer the longer it takes for the target compound to penetrate into the polymer network to interact with the binding sites.

B. Response of the Sensor to Cocaine

The calibration measurements were performed by immersing the probe in different cocaine solutions at various concentrations. The signals were allowed to reach constant values and then recorded. After each measurement, the probe was washed with $\text{MeOH}-\text{AcOH}$ (8:2, v/v) in an ultrasonic bath, followed by the same procedure with MeOH alone to remove bound cocaine. Initially, experiments were carried out in $\text{MeCN}/\text{H}_2\text{O}$ 9:1. MeCN was used because the MIP was prepared in MeCN , so its recognition properties would be expected to be best in MeCN (since this should result in no loss of selectivity due to MIP swelling) [27]. H_2O was added at 10% (v/v) in order to reduce non-specific binding. The sensor exhibited an increase in fluorescence intensity with increasing cocaine concentration in the range from 0 – 250 μM (Fig. 5(a)). At higher concentrations of cocaine, no further change of intensity was observed due to the saturation of all available binding sites. It was also interesting to see if the sensor could work in aqueous media where biological recognition mainly occurs. Measurements were carried out in a manner similar to those of Fig. 5(a) but the solvent system was replaced by $\text{H}_2\text{O}/\text{MeCN}$ 9:1 (MeCN was added to solubilize the analyte). The sensor showed a greater increase in fluorescence in the aqueous than in the organic solution (Fig. 5(b)), which is attributed to the difference between the photophysical properties of the fluorophore in aqueous and in organic media. The dynamic response range of the sensor in aqueous solution is also wider, from 0 up to 500 μM . This arises because non-covalent interactions between cocaine and the functional groups in the MIP were weaker in H_2O and thus the available binding sites were not fully occupied until higher concentrations of cocaine were used. The lower limit of detection of the system may vary since it depends on the type and sensitivity of detector used. With the Ocean Optics

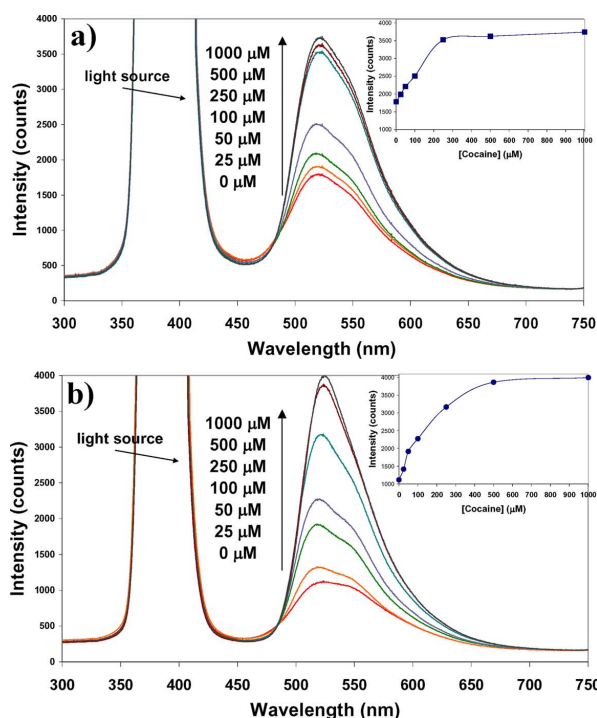


Fig. 5. Response of the sensor to cocaine in the concentration range from 0 to 1000 μM in a) MeCN/H₂O 9:1 and b) in H₂O/MeCN 9:1. Insets show the dependence of emission maximum on cocaine concentration.

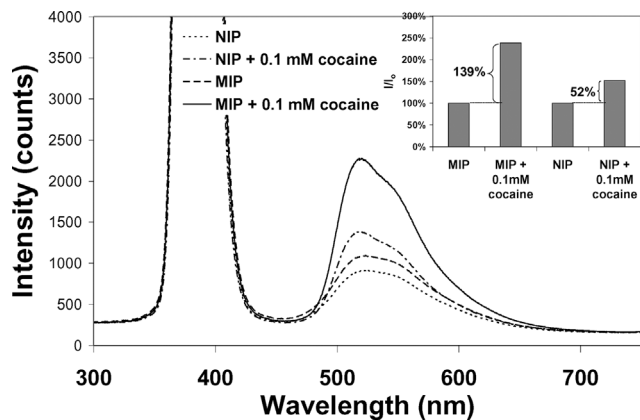


Fig. 6. Response of the sensor probe and control probe to 0.1 mM cocaine in H₂O/MeCN 9:1.

mini-spectrometer used in this work, the lowest concentration of cocaine that can cause a distinguishable change in fluorescence intensity is 2 μM . The response of the control probe (NIP) to cocaine was also studied and it was observed that the NIP probe showed a lesser increase in fluorescence upon cocaine addition of 0.1 mM H₂O/MeCN 9:1 than do the MIP probe (139% compared to 52%, Fig. 6), suggesting that the analyte bound to the MIP more strongly than to the NIP and confirming the existence of recognition sites in the MIP.

C. Selectivity of the Sensor Towards Different Drugs

Different drugs including cocaine, ketamine, amphetamine sulphate, ecgonine methyl ester and buprenorphine.HCl were used for an investigation into the selectivity of the probe developed to cocaine, as it is often seen in the presence of other

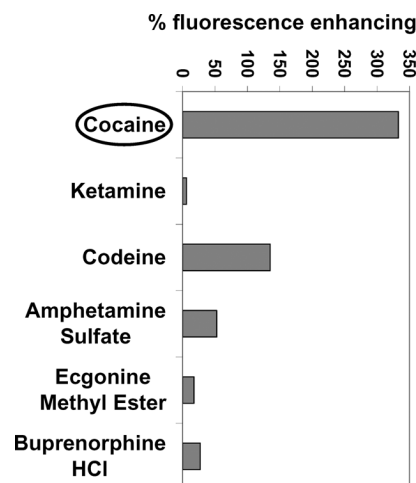


Fig. 7. Response of the sensor probe to different drugs with concentrations of 500 μM in H₂O/MeCN 9:1.

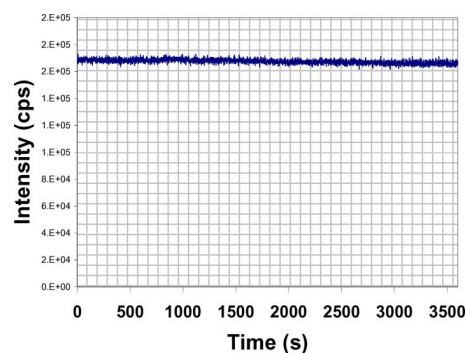


Fig. 8. Fluorescence intensity of the probe at the emission wavelength as a function of time during 60 min of continuous illumination by a high power Xe lamp.

agents. The concentration of all the drugs considered was fixed at 500 μM in H₂O/MeCN 9:1 where the most significant increase in the fluorescence signal intensity was seen for cocaine. It can thus be observed from Fig. 7 that the sensor responds less to any of these drugs than to the template cocaine. This once again indicates successful imprinting and selective recognition sites in the MIP. The difference in fluorescence response of the sensor to different competitors can be explained in terms of the difference in their basicities and the similarity in shape and functional groups of their structures to that of cocaine. Significantly higher reactivity of the sensor for codeine compared to that for other competitors may also be due to the availability of more functional groups on the codeine molecule which are able to interact non-covalently with the binding sites in the MIP. It should also be noted that some of the drugs tested were in the salt forms, not free bases, and the presence of acids might affect the test results obtained.

D. Reproducibility and Photostability

The stability of the probe both in terms of storage, its susceptibility to error due to intense irradiation of the sample and its reproducibility in use is very critical to the successful application of the system. A preliminary evaluation of these parameters was made in order to understand better the performance

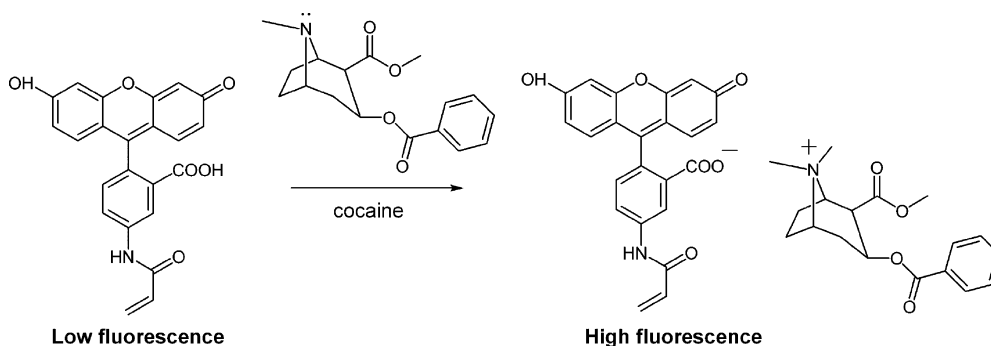


Fig. 9. Interaction between AAF and cocaine.

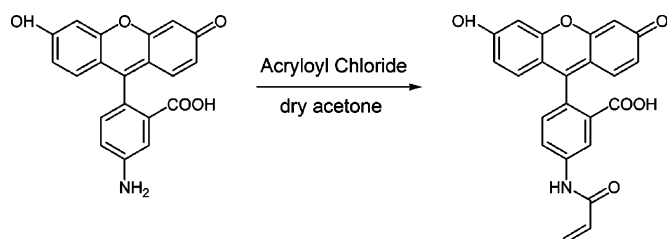


Fig. 10. Preparation of Acrylamidofluorescein (AAF).

of the sensor. The stability of the sensor was tested by calibrating it with different cocaine concentrations ranging from 0 to 500 μM and recalibrating it after 24 h and then one month. After each calibration, the probe was washed thoroughly with MeOH-AcOH (8:2, v/v) in an ultrasonic bath, followed by the same procedure with MeOH alone to remove bound cocaine and then it was stored in the dark until next use. No significant difference was observed between the measurements and the results obtained were found to be fairly reproducible even after one month (data not shown). In order to test the photostability of the sensor, it was coupled into the fluorimeter through a dichroic mirror using a fiber bundle. The excitation light at 375 nm was launched to the distal end of the probe consisting of the sensing material by the high power Xe lamp of the fluorimeter continuously for 1 h. The fluorescence intensity of the probe was dynamically collected. As can be seen from Fig. 8, very little photobleaching (less than 1%) was observed over the time investigated. Compared to the decrease in fluorescence intensity by 65% observed for carboxyfluorescein or by 10–13% observed for iminocoumarin derivatives on their free forms in solution after 60 min of continuous illumination using a mercury lamp [28], the MIP prepared in this work possesses superior photostability, a feature that is critically important with excitation by high intensity solid state sources.

IV. CONCLUSION

In this paper, an effective approach to the development of a sensor for cocaine, showing superior performance and fast response has been reported. The novel robust, compact and portable system developed has been evaluated and preliminary results reported. The sensor have showed an increase in fluorescence intensity in response to cocaine in the concentration range of 0 – 500 μM in aqueous acetonitrile mixtures with good selectivity and reproducibility over one month. Once

its performance is further refined, this type of sensor will potentially make a significant impact on the homeland security enhancement as it can provide technical evidence on the spot with minimum invasion.

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