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Citation: Wren, S., Nguyen, T.H., Gascoine, P., Lacey, R., Sun, T. & Grattan, K. T. V. (2014). Preparation of novel optical fibre-based Cocaine sensors using a molecular imprinted polymer approach. Sensors and Actuators, B: Chemical, 193, pp. 35-41. doi: 10.1016/j.snb.2013.11.071

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Preparation of novel optical fibre-based Cocaine sensors using a molecular imprinted polymer approach

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A B S T R A C T

Novel chemical sensors using fibre optic-based techniques for the detection of Cocaine have been developed, utilising molecularly imprinted polymers (MIPs) containing fluorescein moieties as the signalling groups. The fluorescent MIPs were formed and covalently attached to the distal end of specially chosen optical fibres to create fibre optic probe-based sensors. These sensors exhibited are producible and quantifiable change in the intensity of the fluorescence signal received from the sensor in response to Cocaine in aqueous acetonitrile mixtures. High selectivity for Cocaine over Codeine and a range of known Cocaine interferants has been demonstrated for one of the sensors developed in this work.

1. Introduction

Illicit use of Cocaine is a global problem with world-wide annual Cocaine consumption currently standing at around 600 tonnes. The United Nation's 2010 World Drug Report concluded that the North American Cocaine market alone was valued at $38 billion in 2008 and has been rising since. The World Health Organisation estimated that 0.7% of the global burden of disease in 2004 was due to Cocaine and opioid use, with the social cost of illicit substance use nearly 2% of Gross Domestic Product in those countries that have measured it. Consequently, sensitive and accurate detection of Cocaine is critically important for law enforcement and clinical diagnostics across the world. In 2010, for example, the UK Border Agency made more than 1200 individual seizures of Class A drugs totalling 3000 kg.

The detection of Cocaine has been extensively investigated due to the adverse health effects and related dangers associated with its use [1,2] and *highly sensitive* detection of Cocaine is critically important, as discussed above. Existing technologies for drug detection include manual handling, a method that is rendered less effective as it is easy to miss concealed items during searches and its operation is very time-consuming and resource-intensive. Employment of sniffer dogs involves a high cost (due to the need for specially trained handlers) and is complicated by limited duty cycles and false alarms. Other competing technologies are encumbered by issues such as low sensitivity and poor selectivity and the use of either bulky or fragile systems. These options include Raman Spectrometry [3] (involving the use of sophisticated lasers with high associated expense), Ion Mobility Spectrometry [4] (often gives false alarms), Fourier Transform Infrared Spectroscopy (high false alarm rate), Gas Chromatography–Mass Spectrometry [5] and Liquid Chromatography–Mass Spectrometry (where samples require testing at remote sites and systems are bulky and expensive). In addition, field ID Kits (e.g. drug wipes) make use of expensive consumables and require delicate equipment. Also, many biosensors [6,7] are fragile and costly. Floor standing systems are non-portable and operate over short ranges. Their disadvantages include poor

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effectiveness when moisture is present in air; specific inspection and identification of materials being non-straight forward using Terahertz imaging; high cost and safety concerns (X-ray, Z Backscatter scanning). There is thus a need for new, more reliable and more cost-effective solutions.

Optical fibre sensors can, however, offer many advantages over other sensing technologies. These include their small size, lightweight nature, low cost, the potential for multiplexing multiple sensors on a single fibre network and their remote sensing capability. Such sensors are chemically and physically robust and are particularly suitable for working in harsh environments due to their immunity to electromagnetic interference. In addition, utilising the molecularly imprinted polymer (MIP) technique is a key strength because preparation of synthetic molecular receptors allows recognition of any given target molecule. Other advantages of such sensors are their durability, thermal and chemical stability, low cost and long shelf-life plus MIP-based sensing, provides a more stable alternative to biological receptors. Limited sensing solutions for Cocaine detection (e.g. aptamer-based biosensors [8–11]) are in existence but a compact, hand-held monitor utilising stable synthetic molecular receptors does not exist. This set of technologies includes aptamer-based electrochemical detection [8,9], aptamer-coated, piezoresistive, microcantilever-based biosensing [10] and an electrogenerated chemiluminescence aptamer-based approach [11]. In addition, Hans and Sigrist [12] are developing a sensor for detecting Cocaine in saliva and high affinity MIP nanoparticles [13] and extraction MIPs [14] for Cocaine have been reported.

This work discusses a solution developed in the laboratory which combines the advantages of the use of MIPs with those of employing optical fibre sensing. The focus of this research is aimed at the development of a stable, compact and portable sensing system capable of real time drug detection for use, for example, by security staff, e.g. police forces or airport customs staff in situ to provide a quantitative indication that suspicious materials do contain Cocaine. A rapid method that helps to distinguish between illicit substances and legal impurities (interferants) is also a desirable aim of the sensors developed. Furthermore, the findings disclosed herein do include positive examples of sensor selectivity for Cocaine over Codeine and a range of other masking agents.

2. Technical approach

This research builds on and takes forward previous work by some of the authors [15,16] by enhancement and further optimisation of an approach using a fluorescein-based scaffold interacting with Cocaine. A combination of molecular imprinting, as a method

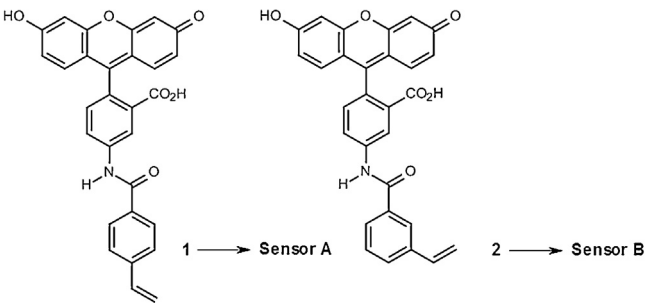

Fig. 2. Chemical structures of fluorophore monomers **1** and **2**.

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 Fig. 1. 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 the target molecules. This imprinting and sensing approach is also illustrated in Fig. 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 and 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.

The strategy employed in this research is designed to enhance the selectivity of the sensor for Cocaine over that reported previously and in relation to other chemical agents – the approach taken thus was two-pronged. Firstly, it was anticipated that additional interactions between the fluorophore and the drug would be achieved through subtle monomer design; however it was recognised that this may not be facile, in light of the distance between the amine group and the two ester groups present in the Cocaine (Fig. 1). A second option involved targeting monomers that bore different functional groups to acrylamidofluorescein [16]. Thus herein, the fabrication of sensors derived from vinylphenyl fluorophores **1** and **2**, as shown in Fig. 2, and labelled sensor **A** and sensor **B** respectively was undertaken and the approach used is described below. Compound **2** was chosen because it is a close analogue of **1** (regioisomer) and could be prepared swiftly.

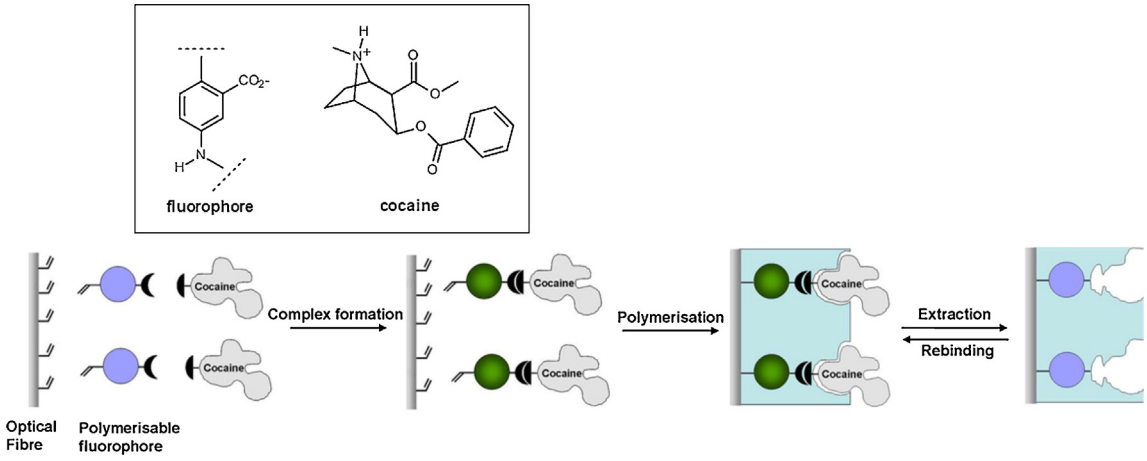

Fig. 1. Fluorescent MIP sensor approach for Cocaine sensing.

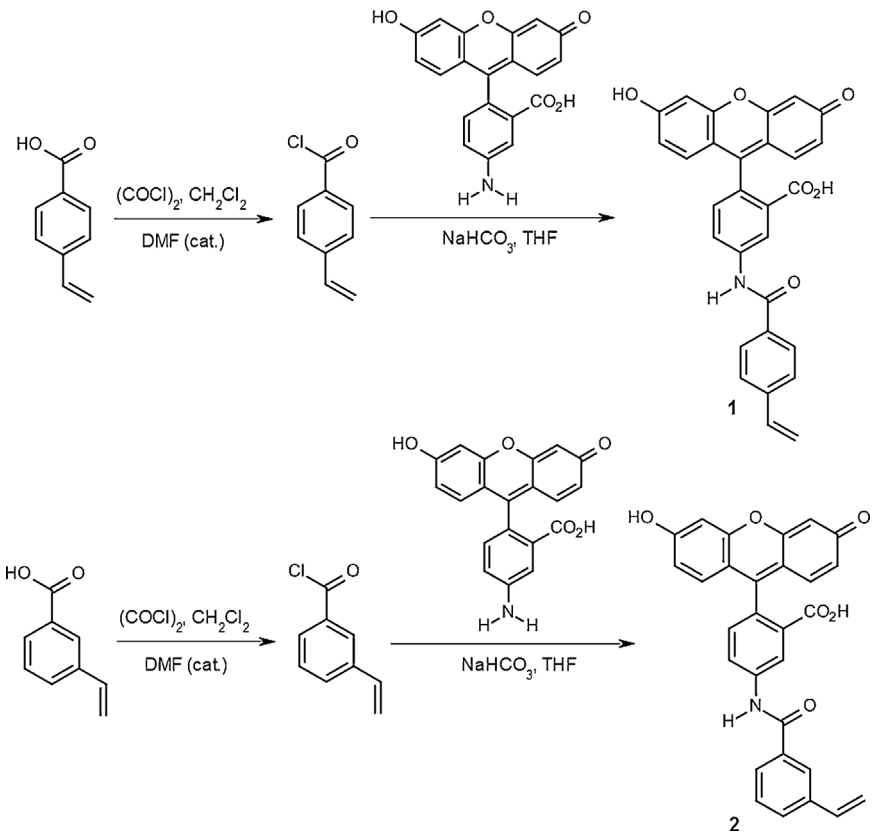

Fig. 3. Synthesis of **1** and **2**.

Synthesis of 1 and 2 was accomplished in 2 steps from 4-vinylbenzoic acid and 3-vinylbenzoic acid respectively, as outlined in Fig. 3. The amide-bond formations using fluoresceinamine were performed using a modified version of the procedure described by Finn et al. [17].

3. Materials and methods

3.1. Apparatus

Mass spectrometry was performed on a Waters LCT Premier Xe electrospray ionisation Time of Flight machine. ^{1}H NMR spectra were recorded on a Bruker Avance instrument operating at 500 MHz. NMR spectra were obtained in solutions of MeOD or DMSO-D6 (reported in ppm). When peak multiplicities are reported, the following abbreviations are used s (singlet), d (doublet), t (triplet), m (multiplet), br (broadened), dd (doublet of doublets). Coupling constants, when given, are reported in Hertz (Hz).

3.2. Reagents

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 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.

3.3. Synthesis of fluorophore monomers

3.3.1. Synthesis of N-[4-(3-hydroxy-6-oxo-xanthen-9-yl)-3-methyl-phenyl]-4-vinyl-benzamide (*1*)

To a stirred mixture of 4-vinylbenzoic acid (222.2 mg, 1.50 mmol) in anhydrous dichloromethane (17 ml) was added DMF (2 drops, catalytic quantity) and oxalyl chloride (285 μl, 3.328 mmol) and the reaction left at room temperature for 1 h. After evaporation of the volatiles in vacuo, the residue (4-vinylbenzoyl chloride) was re-suspended in anhydrous tetrahydrofuran (25 ml). Subsequent addition of fluoresceinamine (9-(4-amino-2-methyl-phenyl)-6-hydroxy-xanthen-3-one; 474 mg, 1.364 mmol) and sodium bicarbonate (247.4 mg, 2.945 mmol) was followed by stirring at room temperature overnight (for 19.5 h in total). The reaction mixture was filtered and the filtrate concentrated to give the crude product. Purification by flash chromatography (Kieselgel 60, eluting with 2% methanol in ethyl acetate followed by 1:1:4:4 methanol:acetone:chloroform:toluene) and trituration from hexane (×2) gave the title compound as an orange solid.

MS (ES−), M−1 476.11; ^{1}H NMR (D_6 DMSO): 10.65 (1H, br), 10.10 (2H, br), 8.49 (1H, m), 8.10 (1H, m), 7.99 (2H, d, J = 10.0 Hz), 7.66 (2H, d, J = 5.0 Hz), 7.26 (1H, d, J = 5.0 Hz), 6.84 (1H, dd, J = 10.0 and 15.0 Hz), 6.66 (2H, m), 6.60–6.62 (2H, m), 6.53–6.56 (2H, m), 6.01 (1H, d, J = 20.0 Hz), 5.42 (1H, d, J = 15.0 Hz).

3.3.2. Synthesis of N-[4-(3-hydroxy-6-oxo-xanthen-9-yl)-3-methyl-phenyl]-3-vinyl-benzamide (*2*)

This compound (above) was prepared using the same method described above (starting from 3-vinylbenzoyl chloride).

MS (ES−), M−1 476.11; ^{1}H NMR (MeOD): 8.36 (1H, br), 8.05–8.10 (2H, m), 7.88 (1H, d, J = 10.0 Hz), 7.68 (1H, d, J = 10.0 Hz),

7.51 (1H, m), 7.22 (1H, m), 6.81–6.90 (3H, m), 6.67 (2H, m), 6.60 (2H, m), 5.94 (1H, d, J = 20.0 Hz), 5.36 (1H, d, J = 10.0 Hz).

3.4. Sensor fabrication

Fabrication of Cocaine-sensitive fibre optic probes (labelled sensor **A** and sensor **B** (Fig. 2)) was achieved, requiring a multi-step process. [15] The distal end of a 1000 μm 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 °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 silanising 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-polymerisation mixture was prepared by dissolving Cocaine (2 equiv.), fluorophore (1 equiv.), ethylene glycol dimethacrylate cross linker (80 equiv.), acrylamide co-monomer (14 equiv.) 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 using melted plastic from a disposable syringe and polymerised in an oven at 70 °C (over 74 h). 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. The sensor tip was washed repeatedly with methanol-acetic acid (8:2, v/v) in an ultrasonic bath, followed by the same procedure with methanol 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) for sensor **A** was also prepared under identical conditions, using the same protocol but without the addition of Cocaine.

3.5. Experimental set-up

The experimental set-up used for the measurements undertaken to calibrate the probe is shown in Fig. 4, where light from a LED, emitting at a centre wavelength of 375 nm, was coupled through a multimode UV/vis fibre 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 fibre coupler. The far end of the coupler, made using two multimode UV/vis fibres with hard polymer cladding, 600 μm 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). The spectrometer's sensitivity of 75 photons/count (at 400 nm) and 41 photons/count (at 600 nm) is sufficient for this study.

The following software settings were selected for sensor **B**; 10 ms integration time, 20 scans to average and 2 point boxcar width. Higher values were chosen when profiling sensor **A** (100 ms integration time, 23 scans to average and 10 point (boxcar width)) in order to allow the detector to monitor incoming photons for longer and to improve the signal-to-noise ratio.

4. Results and discussion

4.1. Response of the sensors to Cocaine

An accurate calibration of the sensors described previously was performed using the 9:1 water:acetonitrile solvent system discussed in previous work by the authors [15]. This was carried out over periods of 15 min (sensor **A**) and 5 min (sensor **B**) respectively. The fact that the sensors attained equilibrium within 15 min compares favourably with other MIP sensor systems [18,19]. In the tests carried out, it was seen that sensor **A** responded to1000 μM Cocaine yet exhibited minimal response at lower concentrations (Fig. 5). The response of the control/NIP (non-imprinted polymer) probe to Cocaine was also studied and no increase in fluorescence (rather an insignificant ~1% decrease) was observed upon addition of 1000 μM Cocaine (at 526 nM) relative to the situation seen when zero Cocaine was present, as shown in Fig. 6b. 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 which confirms the existence of effective MIP recognition in this system.

The fluorescence spectrum for sensor **B** was also measured and these data are also depicted in Fig. 5. The positive response at 500 and 1000 μM of the analyte suggests a larger dynamic range than observed for sensor **A** and further profiling of sensor **B** is ongoing.

4.2. Response of sensor *A* to Codeine

The cross-sensitivity to other substances often used to 'cut' drugs sold illegally for recreational use is important for the development of a monitor to use used by police and security personnel to determine if a banned substance is present in a sample seized for investigation. Thus an investigation of sensor selectivity to commonly used materials is very important. Codeine is one such material – it is cheap, has analgesic properties and can be available as a white powder, thus resembling pure Cocaine and when

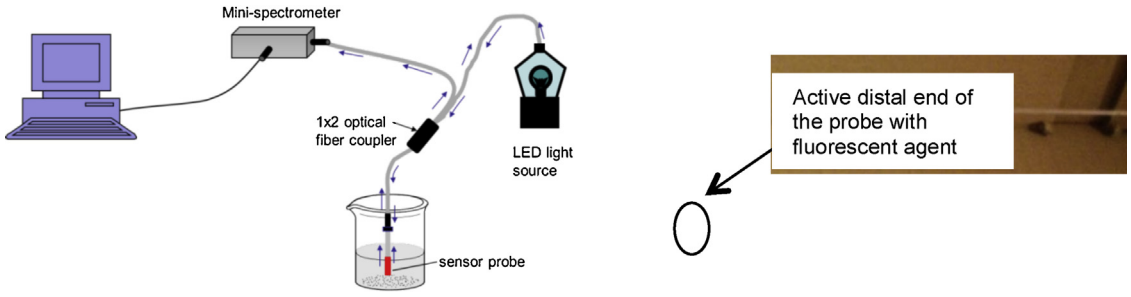

Fig. 4. Sensor system and Cocaine probe derived from **1**. Photograph shows the distal end of the probe.

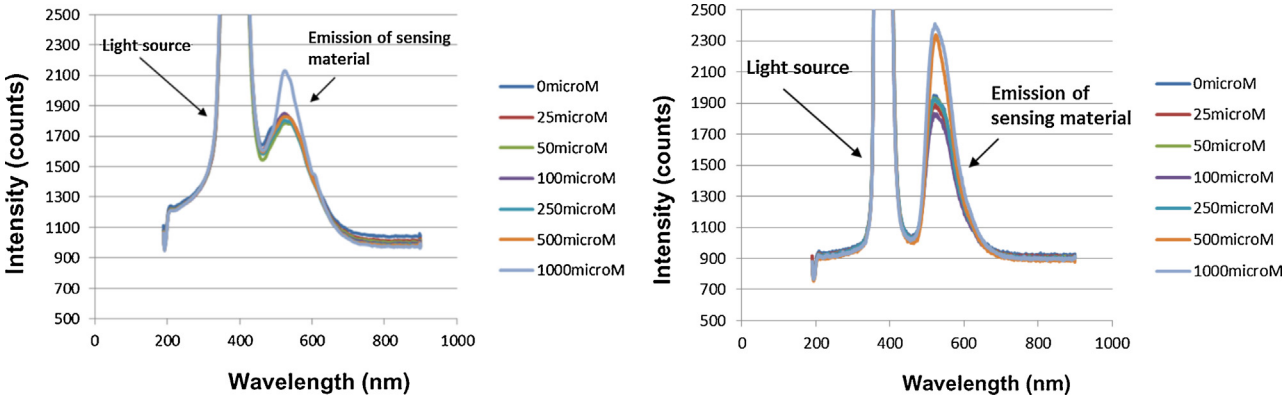

Fig. 5. Fluorescence spectra of sensors **A** (left) and **B** (right).

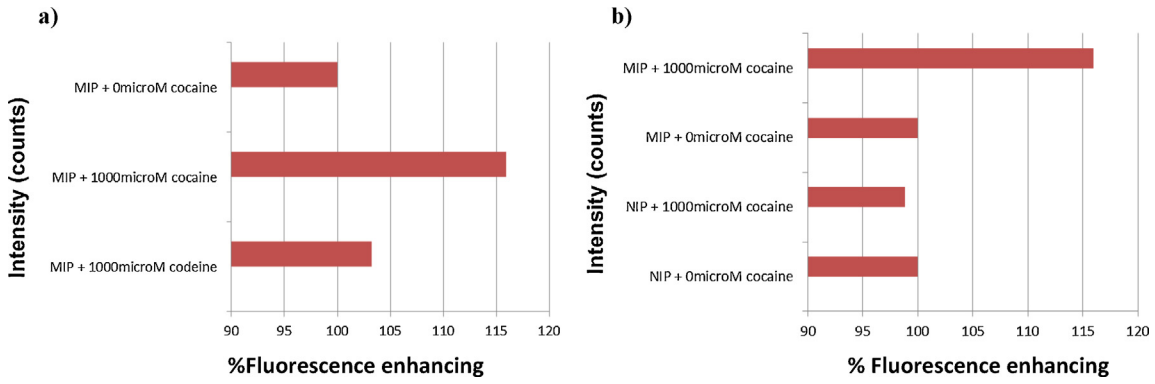

Fig. 6. Measurement results for sensor **A** (all in H_2O:MeCN 9:1). (a) Response to Codeine and Cocaine; (b) response of sensor and control/NIP probe.

compared to other drugs, such a test was deemed important because of this combination with other materials. In our previous studies [15] significantly higher reactivity of the sensor developed for Codeine was observed, when compared to that for other agents, and this is significant for the application under consideration. The sensors developed here show a much better response in light of this issue – a positive indication is seen in Fig. 6a which shows that sensor **A** responds far less to Codeine than does Cocaine (1000 μM, in 9:1 water:acetonitrile; 3% fluorescence enhancement compared to 16%, at 526 nm). Sensor selectivity for **B** is on-going but is expected to show a similar lack of cross-sensitivity to this important chemical.

4.3. *Interferant testing*

Cocaine is often cut with a variety of other substances 'on the street' so further tests were carried out on the response of this sensor design, using sensor **A**, to a representative set of known interferants (these are listed in Table 1). Solutions of the interferants were prepared using concentrations of 0.30 mg/mL (in 9:1 water:acetonitrile; equivalent to 1000 μM Cocaine in weight). The sensor was rinsed with methanol after each measurement. Sensor **A** exhibited a positive fluorescence response to biological washing powder (Persil) but responded less to baby teething powder (Teetha™), bicarbonate of soda, D(−)-fructose and salt compared to Cocaine (see Figs. 8 and 9). The rationale for the fluorescence swamping effect of the biological washing powder is likely to originate from the presence of brightening agents in the washing powder. This does provide a means to minimise this effect as the fluorescence spectrum of such agents can be readily evaluated from measurements made on a range of common washing powders and a narrow spectrum filter used to select the specific spectral peaks in the fluorescence spectrum seen from the active sensor materials (as shown in Fig. 5, for sensors **A** and **B**) and thus to eliminate the majority of the broad peak that is a feature of commonly used brightening agents in commercial washing powders. Spectra of brightening agents from commonly used commercial biological washing powders Persil and Aerial with Actilift™ (using sensor **A** and 0.30 mg/mL solutions in 9:1 water:acetonitrile, at a 375 nm excitation LED) are shown in Fig. 7.

The results of a series of tests using these substances are shown in Fig. 8, for the range of materials discussed in Table 1. The fluorescence spectra from samples containing these interferants are shown in Fig. 8 and Fig. 9 depicts the results relative to Cocaine at a specific wavelength.

Further optimisation of this test profile is planned by separation of the fluorescence spectral traces arising from Cocaine and interferants and expansion of the interferant screening set is also

Table 1
List of common interferants considered in this work.

Interferant	Key components
Biological washing powder (Persil)	5–15% anionic surfactants, oxygen-based bleaching agents, less than 5% soap, perfume, brighteners, enzymes, phosphonates
Baby teething powder (Teetha™, Nelsons)	Homoeopathic Chamomilla, lactose, xylitol and starch
Bicarbonate of soda	Sodium hydrogen carbonate used as a pure substance
D(−)-Fructose	Used as a pure substance from commercially available samples
Salt	Used as a pure substance from commercially available samples

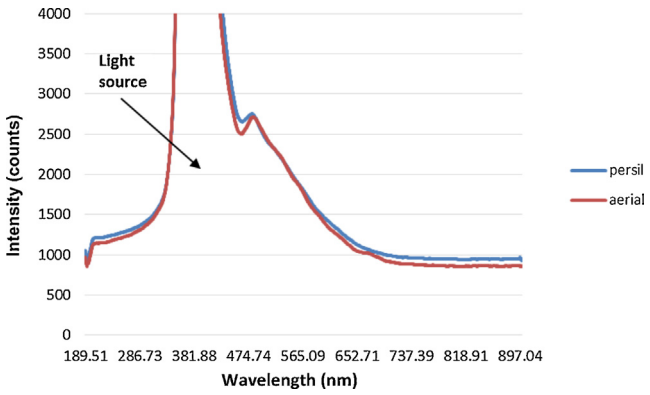

Fig. 7. Fluorescence spectra of brand-leading biological washing powders often seen as interferants.

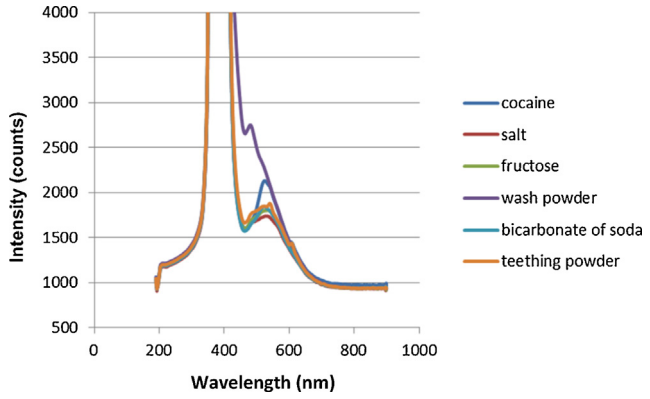

Fig. 8. Interferant test results for sensor **A** – fluorescent spectra of substances shown.

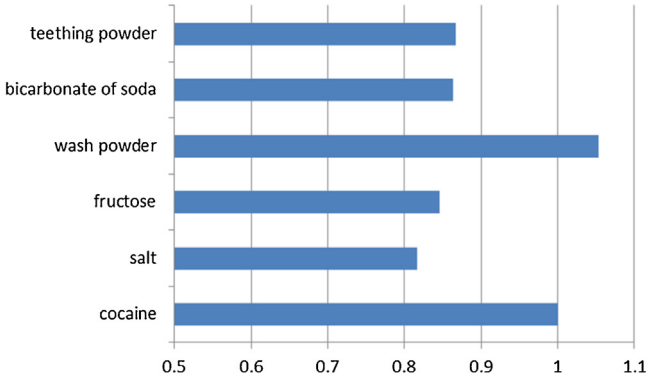

Fig. 9. Alternative depiction of interferant test results (sensor **A**). Fluorescence intensity relative to Cocaine in arbitrary units (horizontal axis) using a wavelength of 526 nm.

planned. The main effort will be focused on the optimisation of the probe designs, based on the promising results obtained above.

5. Conclusions

In this paper, the on-going development of chemical sensors for the monitoring of 'street' Cocaine in samples such as those examined or seized by the security forces is reported. Two different sensor designs using novel compounds, specially developed and synthesised for use at the distal end of a fibre optic probe were investigated and results on their performance reported. The sensors showed very good performance through an increase of fluorescence intensity in response to Cocaine at 500 and 1000 μM in an aqueous acetonitrile mixture. For sensor **A**, promising selectivity for Cocaine over Codeine was also demonstrated and a very satisfactory performance in terms of its response in the interferant test profile.

Further refinement of drug sensors of this type is currently underway and will be reported on in due course. Improvement of the performance of sensors such as these involves the variation and the optimisation of the polymerisation conditions and the use of advanced computational methods to design and engineer appropriate new sensor materials. This will lead to the fabrication of further sensor designs which will be evaluated for sensitivity and selectivity in response to other agents and evaluation of their performance in light of such issues as repeatability and photostability allowing devices to be available to be tested in the field. The lightweight, low-power and non-electrical nature of the fibre optic sensor approach is ideally suited to use by security officers for evaluations where they work on in the field, prior to more extensive laboratory evaluations.

Thus the approach shows importance for sensing additional drugs of concern and that this work has significant potential for commercial applications in the homeland security field.

Acknowledgements

The authors would like to thank the support from the Engineering and Physical Sciences Research Council, Home Office CAST and Smiths Detection. The support of the George Daniels Educational Trust is greatly appreciated.

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Biographies

Stephen P. Wren received his BSc degree in Chemistry from Manchester University (1992) and his PhD in Organic Synthesis from Cambridge University (1995). After working as a postdoctoral scientist at the University of Texas at Austin (1996–1997), he spent 15 years in industry developing and commercialising novel medicines. He is the author/inventor more than 50 publications/patents. Currently, he is a Research Fellow in Chemical Sensing at City University London working in the group headed by Professor Tong Sun and Professor K.T.V. Grattan. His research involves designing and fabricating optical fibre sensors with important commercial applications.

T. Hien Nguyen received her BSc degree in chemistry from Vietnam National University, Hanoi, Vietnam, in 2002. She obtained the MSc degree and the PhD degree in chemistry in 2003 and 2007 respectively, from the University of Leeds, UK. Afterwards, she became a Research Fellow in advanced chemical sensing in the School of Engineering and Mathematical Sciences, City University, London, UK until 2012. She is currently working as a Research Fellow at the Organic Semiconductor Centre, University of St Andrews, UK. Her research interests are in the field of molecular recognition, molecularly imprinted polymers, conjugated polymers and chemical sensors.

Paul Gascoine studied part time for an M.I.Biol (Chartered biologist) qualification and then went on to gain a PhD from Royal Free Hospital School of Medicine (London University) for investigations into aqueous polymer two phase systems and their interaction with cell membranes. After post-doctoral work studying nephrotic syndrome in children (Great Ormond St Hospital) and the effect of tPA (tissue plasminogen activator) in fibrinolysis (National Institute for Biological Standards and Control) he moved to Amersham international (now GE). At Amersham Paul's work involved the development of in vitro diagnostic kits for the determination of HIV and Hepatitis. This included some Cat III work with the live agents, testing positive blood samples. Since 1999 he has been employed at Smiths Detection near Watford involved in numerous biodetection programmes for both UK MoD and overseas governments. Currently his roles include supporting the ongoing programmes, technical sales support and investigating new technologies for possible inclusion in forthcoming Smiths products.

Richard Lacey has a first degree in Physics and a PhD in Solid state Physics from Queen Mary, London. He is a chartered Physicist and a fellow of both the Royal Society of Chemistry and the Institute of Physics. He has been in the Home Office since 1979 and has had a number of roles. Recently he has been working in the field of Contraband detection having previously been the chief scientist for adding drugs, money and people smuggling to the portfolio. His current responsibilities include strategic futures, academic engagement and the Centre for Applied Science and Technology's (CAST) innovation portfolio. He has a visiting chair in the Jill Dando institute at UCL.

Tong Sun was awarded the degrees of Bachelor of Engineering, Master of Engineering and Doctor of Engineering for work in mechanical engineering from the Department of Precision Instrumentation of Harbin Institute of Technology, Harbin, China, in 1990, 1993 and 1996, respectively. She came to City University, London, as an Academic Visitor and latterly a research fellow to work in the field of fibre optic temperature measurement using luminescent techniques. She was awarded the degree of Doctor of Philosophy at City University in applied physics in 1999. She was an assistant Professor at Nanyang Technological University in Singapore from 2000 to 2001 and is currently Professor of Sensor Engineering at City University, London, since she re-joined in April 2001. Professor Sun is a Member of the Institute of Physics and the Institution of Electrical Engineers and a Chartered Physicist and a Chartered Engineer in the United Kingdom. Her research interest is in optical fibre sensors, optical communications and laser engineering. She has authored or co-authored well over 100 scientific and technical papers in the field.

Kenneth T.V. Grattan received his Bachelors degree in physics from The Queen's University, Belfast, in 1974 and completed his PhD studies in 1978, graduating from the same University. In the same year he became a post-doctoral research assistant at Imperial College, London. His research during that period was on laser systems for photophysical systems investigations, and he and his colleagues constructed some of the first of the then new category of excimer lasers (XeF, KrF) in Europe in 1976. His work in the field continued with research using ultraviolet and vacuum ultraviolet lasers for photolytic laser fusion driver systems and studies on the photophysics of atomic and molecular systems. He joined City University, London in 1983 after 5 years at Imperial College, undertaking research in novel optical instrumentation, especially in fibre optic sensor development for physical and chemical sensing. The work has led into several fields including luminescence based thermometry, Bragg grating-based strain sensor systems, white light interferometry, optical system modelling and design and optical sensors for water quality monitoring. The work has been extensively published in the major journals and at international conferences in the field, where regularly he has been an invited speaker, and over 700 papers have been authored to date. Professor Grattan is currently Dean of the City Graduate School.