Wren, S., Sun, T. & Grattan, K. T. V. (2015). A Fluorescent Optical Fibre Chemosensor for Mercury Detection. 24th International Conference on Optical Fibre Sensors, 9634, 96346C. doi: 10.1117/12.2194588

# Fluorescent optical fibre chemosensor for the detection of mercury

T. Hien Nguyen*, Stephen P. Wren, Tong Sun and Kenneth T.V. Grattan
Photonics and Instrumentation Research Centre, City University of London, Northampton Square, London, EC1V 0HB, UK

## ABSTRACT

This work aims to develop a stable, compact and portable fibre optic sensing system which is capable of real time detection of the mercury ion (II), $Hg^{2+}$. A novel fluorescent polymeric material for $Hg^{2+}$ detection, based on a coumarin derivative (acting as the fluorophore) and an azathia crown ether moiety (acting as the mercury ion receptor), has been designed and synthesized. The material was covalently attached to the distal end of an optical fibre and exhibited a significant increase in fluorescence intensity in response to $Hg^{2+}$ in the μM concentration range *via* a photoinduced electron transfer (PET) mechanism. The sensor has also demonstrated a high selectivity for $Hg^{2+}$ over other metal ions. A washing protocol was identified for sensor regeneration, allowing the probe to be re-used. The approach developed in this work can also be used for the preparation of sensors for other heavy metals.

**Keywords:** Optical fiber sensor, chemosensor, mercury sensor, fluorescent sensor, photoinduced electron transfer

## 1. INTRODUCTION

Mercury pollution in soil due to mining and industrial activities poses a serious problem across the world both from an economic and health perspective[1-3]. Common methods for the remediation of mercury-contaminated soil include excavation and disposal but these methods are often costly and crude. Mercury, both in the inorganic form as $Hg^{2+}$ or in the organic form as methyl mercury, once introduced into the body, can accumulate and cause serious irreversible damage to the immune system, the central nervous system and kidneys[2, 4]. It is also believed that mercury causes various neurodegenerative diseases such as Alzheimer's diseases and Parkinson's disease[3]. Therefore, the detection of mercury is very important for the protection of human health and the minimization of its exposure in the environment is critical. Such sensors would also provide a warning of exposure and thus act as a trigger for treatment.

In recent years, a number of chemical sensors for the detection of $Hg^{2+}$ have been reported, based either on electrochemical methods[5-7] or fluorescence[8-20]. However, there are weaknesses with them and their applications, where most are not suitable for use in the field. Biosensors based on whole bacterial cells or bacterial heavy metal binding proteins [21, 22], which can be considered as alternative devices, also suffer from certain limitations due to the fragile and unstable nature of the biological recognition elements. Commercial heavy metal sensors for use in soil are very limited and are typically either very expensive or require the extraction of soil prior to its manipulation and analysis, which could allow sample degradation. Consequently, there is a strong industrial need for the development of a low-cost and portable alternative for mercury detection, thus providing a fast screening solution to yield new information on what is an important aspect of improving the environment.

This work aims to develop a stable, compact and portable fibre optic sensing system which is capable of real time detection of the mercury ion (II). The fibre optic approach used here is familiar for the advantages offered over conventional means, in terms of small size, immunity to electromagnetic interference, remote sensing capability, resistance to chemicals and biocompatibility[23]. A novel fluorescent polymeric material for $Hg^{2+}$ detection based on a derivative of coumarin (acting as the fluorophore) and an azathia crown ether moiety (acting as the mercury ion receptor) has been designed, synthesized and covalently attached to the distal end surface of an optical fibre. Azathia crown ethers have been reported to have strong ability to coordinate with heavy and transition metal ions and have previously been used as receptors in the design of fluorescent sensors for $Hg^{2+}$ [9, 24]. However, the signalling mechanism employed in those sensors was based on intramolecular charge transfer (ICT), leading to the quenching of fluorescence upon analyte

*hien.nguyen@city.ac.uk; phone +44 (0)20 7040 3257; fax +44 (0)20 7040 8568

binding. It is strongly desirable that the fluorescence modulation is in the 'off-on' direction, since this will lead to the best signal to noise characteristics and avoid potential false positive responses due to degradation of the system. In this sensor design, the presence of the amine significantly reduces the fluorescence of the fluorophore due to the quenching of its fluorescence by the nitrogen lone pair electrons through PET. Upon complex formation with $Hg^{2+}$, the nitrogen lone pair electrons are donated to $Hg^{2+}$, which therefore abolishes or tremendously reduces the fluorescence quenching. Consequently, binding of $Hg^{2+}$ switches on fluorescence as illustrated in Fig.1.

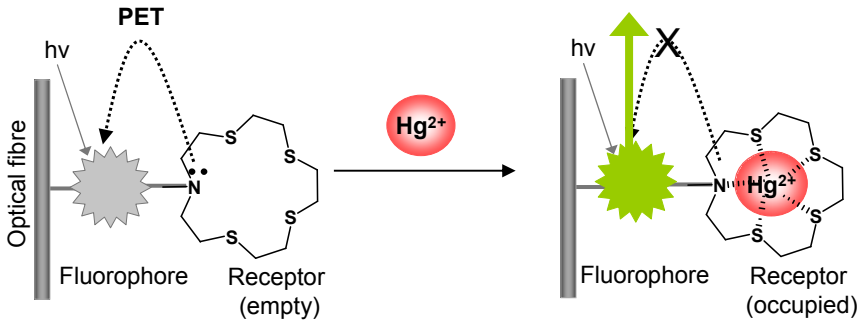


Figure 1. Illustration of fluorescence switching on by $Hg^{2+}$ binding *via* a PET mechanism.

## 2. EXPERIMENTAL

### 2.1 Sensor fabrication

The fabrication of the $Hg^{2+}$ sensing probe required a multi-step process, using a novel polymerisable coumarin dye bearing an azathia crown ether moiety (CACE). CACE absorbs at 342 nm and emits at 471 nm in $H_2O$/MeCN (7:3, v/v) (Fig. 2a). It shows a significant increase in fluorescence intensity in response to $Hg^{2+}$ in an aqueous acetonitrile mixture (Fig.2b). CACE was covalently bound to the optical fibre by copolymerizing with methacrylic acid and ethylene glycol dimethacrylate (EDMA) cross linker using 2,2-dimethoxy-2-phenyl-acetophenone as the initiator on the surface of the fibre that was prepared and functionalized with polymerizable methacrylate groups following the previously reported method[25]. The whole process was carried out in an argon filled glove box by alternately immersing the fibre tip in the pre-polymerization mixture using a dip coater and curing with 365 nm UV radiation (3 times). The sensor tip was washed in methanol and distilled water to remove all unreacted materials and the excess amount of polymer formed which was not directly bound to the fibre. The probe was then stored in a cool and dark place until its later use.

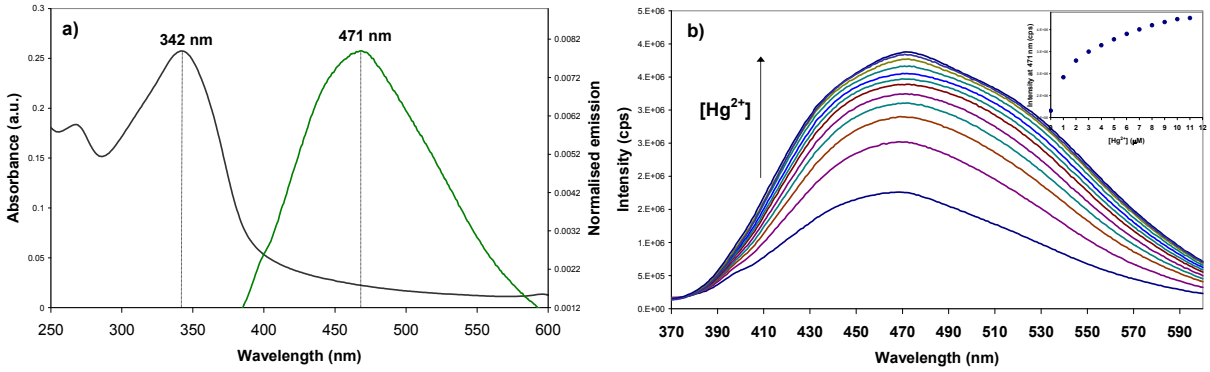


Figure 2. a) Absorption and emission spectra of CACE (10 µM) in $H_2O$/MeCN (7:3, v/v). Emission spectrum recorded with λex = 350 nm. b) Emission spectra of CACE at [$Hg^{2+}$] ranging from 0 to 11 µM. The inset shows the titration plots at 471 nm (λex = 350 nm).

### 2.2 Experimental set-up

The set-up used for the measurements undertaken to evaluate the performance and thus 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 multimode UV/Visible fibre, using collimation and focusing lenses, into one branch of a 2x1 multimode fibre coupler. The other end of the fibre coupler is connected to the sensor probe with the active sensing region being located at the distal end of the fibre. Following the interaction of $Hg^{2+}$ with the active region, a portion of the total light emitted from the sensing layer is collected and guided through the other branch of the fiber coupler to an Ocean Optics USB2000 spectrometer, with the output being displayed on a computer screen.

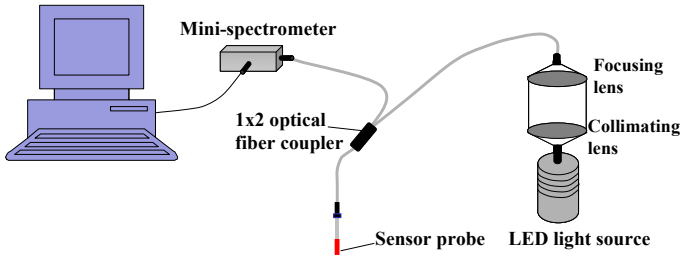


Figure 3. Experimental set-up used in the evaluation of the performance of the probe designed.

## 3. RESULTS AND DISCUSSION

### 3.1 Response of the sensor to $Hg^{2+}$

The calibration of the sensor was performed using a series of solutions of mercury chloride in deionized water. The probe was immersed in the $Hg^{2+}$ solutions and the signals were allowed to reach constant values (~ 2 minutes) before being recorded. The sensor was rinsed with deionized water between measurements. In a way that is similar to that seen for the free dye, the sensor exhibited an increase in fluorescence intensity with increasing $Hg^{2+}$ concentration in the range of 0 - 14 µM (Fig. 4). At higher concentrations of $Hg^{2+}$, no further change of intensity was observed due to the saturation of all available binding groups. It has also been noted that the emission peak of the immobilized form of the dye is slightly 'red shifted' compared to that of its free form in solution – that is to a longer wavelength. It seems that this probably can be attributed to the change in the polarity of the microenvironment.

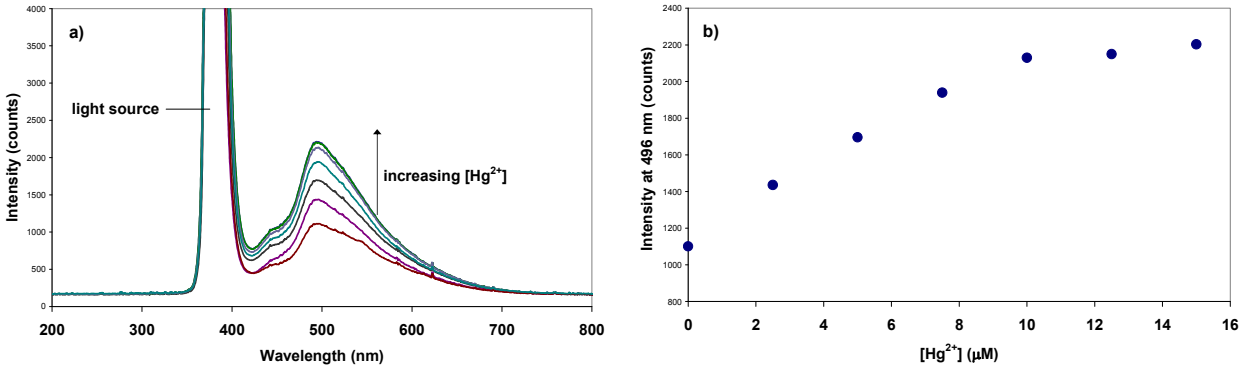


Figure 4. a) Fluorescence spectra of the sensor probe in deionised water with the addition of $Hg^{2+}$ b) Plot showing the change of fluorescence intensity with changing $Hg^{2+}$ concentration.

### 3.2 Photostability and Reusability

Photostability is one of the critical properties of fluorescent materials and thus of the fluorophore used in this sensor application. In order to test the photostability of the fluorophore, the probe was coupled into the fluorimeter through a dichroic mirror using a fibre bundle. The excitation light (at a wavelength of 350 nm) was launched to the distal end of the probe illuminating the sensing material with light from the intense, high power Xe lamp of the fluorimeter continuously for 1 h. The fluorescence intensity data from the probe were collected over that period and displayed. As can be seen from Fig. 5, very little photobleaching (less than 1%) was observed over the time investigated and with the high flux of photons onto the probe, indicating that the material prepared possesses excellent photostability, a feature that is critically important with excitation of sensor probes by high intensity solid state sources.

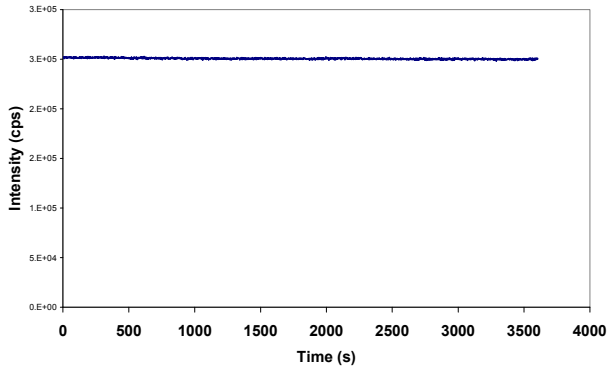


Figure 5. Fluorescence intensity of the probe at the emission wavelength as function of time during 60 min of continuous illumination by light from a high power Xe lamp.

Sensor reusability is important for the development of a tool that can allow multiple, rapid and real-time measurements in the field. Consequently, a range of known mercury binders and washing agents were screened to identify a method for probe regeneration. It was found that TPEN (N,N,N′,N′-tetrakis(2-pyridylmethyl)ethylenediamine) proved superior to EDTA and nitric acid. The spectral comparison before and after the TPEN incubation with a return of fluorescence indicates that the probe can be regenerated by removal of mercury (II) ions using 10 mM TPEN in deionized water, and subsequent rinsing with deionized water, for multiple measurement cycles. Further optimization of this protocol is in on-going to refine the sensor design and packaging for the next-stage field tests.

### 3.3 Selectivity of the sensor towards other cations

The responses of the sensor probe to the presence of various biologically and environmentally relevant metal ions were investigated and the results are illustrated in Fig. 6. The concentration of all the ions tested was fixed at 10 μM where a significant increase in fluorescence intensity was seen for $Hg^{2+}$. The fluorescence profiles of the probe were almost unchanged in the presence of $Pb^{2+}$, $Cu^{2+}$, $Cd^{2+}$, $Ni^{2+}$, $Ca^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ indicating excellent selectivity for $Hg^{2+}$ over these cations and a very important result for the in the field use of a sensor like this where such cross contamination is likely.

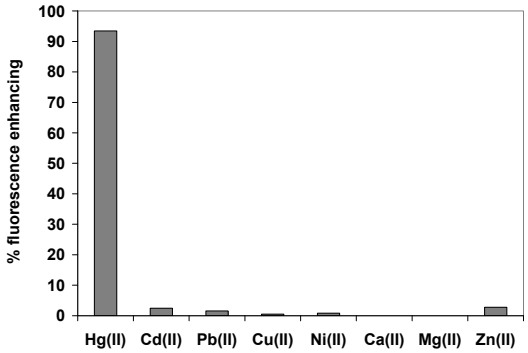


Figure 6. Response of the sensor probe to a range of other cations (10 μM).

## 4. CONCLUSIONS

A stable, compact and portable optical fibre sensor able to detect mercury in dilute aqueous solutions has been prepared, evaluated and preliminary results reported. The sensor has showed an increase in fluorescence intensity in response to $Hg^{2+}$ in the μM concentration range *via* a photoinduced electron transfer mechanism with excellent selectivity over relevant metal ions. Transfer to field testing in soil may require hydration of the sensor tip, depending on ground conditions and appropriate sensor 'packaging' to withstand use by inexperienced operators. With on-going work to refine the performance, this type of sensor has the potential to be an important tool for better environmental monitoring.


## ACKNOWLEDGEMENT

The authors would like to thank the financial support from the EU to enable the development of a suite of chemical sensors at City University of London for identification of soil pollutants under the Pollins (Automated Pollution Inspection Scanning System for Soil using a robotic vehicle) project.


## REFERENCES


[1] Wang, J. X., Feng, X. B., Anderson, C. W. N., Xing, Y., and Shang, L. H., "Remediation of mercury contaminated sites - A review," *J. Hazard. Mater.*, vol. 221, 1-18 (2012).

[2] Holmes, P., James, K. A. F., and Levy, L. S., "Is low-level environmental mercury exposure of concern to human health?," *Sci. Total Environ.*, vol. 408, 171-182 (2009).

[3] Mutter, J., Naumann, J., Sadaghiani, C., Walach, H., and Drasch, G., "Amalgam studies: Disregarding basic principles of mercury toxicity," *Int. J. Hyg. Environ. Health.*, vol. 207, 391-397 (2004).

[4] Houston, M. C., "Role of Mercury Toxicity in Hypertension, Cardiovascular Disease, and Stroke," *J. Clin. Hypertens.*, vol. 13, 621-627 (2011).

[5] Liu, Z. H., Huan, S. Y., Jiang, J. H., Shen, G. L., and Yu, R. Q., "Molecularly imprinted TiO2 thin film using stable ground-state complex as template as applied to selective electrochemical determination of mercury," *Talanta*, vol. 68, 1120-1125 (2006).

[6] Bui, M. P. N., Brockgreitens, J., Ahmed, S., and Abbas, A., "Dual detection of nitrate and mercury in water using disposable electrochemical sensors," *Biosens. Bioelectron.*, vol. 85, 280-286 (2016).

[7] Noh, M. F. M. and Tothill, I. E., "Determination of Lead(II), Cadmium(II) and Copper(II) in Waste-Water and Soil Extracts on Mercury Film Screen-Printed Carbon Electrodes Sensor," *Sains Malays.*, vol. 40, 1153-1163 (2011).

[8] Nolan, E. M. and Lippard, S. J., "Turn-on and ratiometric mercury sensing in water with a red-emitting probe," *J. Am. Chem. Soc.*, vol. 129, 5910-5918 (2007).

[9] Isaad, J. and El Achari, A., "Azathia crown ether possessing a dansyl fluorophore moiety functionalized silica nanoparticles as hybrid material for mercury detection in aqueous medium," *Tetrahedron*, vol. 69, 4866-4874 (2013).

[10] Aydin, Z. Y., Wei, Y. B., and Guo, M. L., "An "off-on" optical sensor for mercury ion detection in aqueous solution and living cells," *Inorg. Chem. Commun.*, vol. 50, 84-87 (2014).

[11] Bera, K., Das, A. K., Nag, M., and Basak, S., "Development of a Rhodamine-Rhodanine-Based Fluorescent Mercury Sensor and Its Use to Monitor Real-Time Uptake and Distribution of Inorganic Mercury in Live Zebrafish Larvae," *Anal. Chem.*, vol. 86, 2740-2746 (2014).

[12] Dai, H. L. and Xu, H., "A water-soluble 1,8-naphthalimide-based 'turn on' fluorescent chemosensor for selective and sensitive recognition of mercury ion in water," *Bioorg. Med. Chem. Lett.*, vol. 21, 5141-5144 (2011).

[13] Hou, C., Urbanec, A. M., and Cao, H. S., "A rapid Hg2+ sensor based on aza-15-crown-5 ether functionalized 1,8-naphthalimide," *Tetrahedron Lett.*, vol. 52, 4903-4905 (2011).

[14] Kaewtong, C., Niamsa, N., Wanno, B., Morakot, N., Putpoka, B., and Tuntulani, T., "Optical chemosensors for Hg2+ from terthiophene appended rhodamine derivatives: FRET based molecular and in situ hybrid gold nanoparticle sensors," *New J. Chem.*, vol. 38, 3831-3839 (2014).

[15] Kaewtong, C., Wanno, B., Uppa, Y., Morakot, N., Pulpoka, B., and Tuntulani, T., "Facile synthesis of rhodamine-based highly sensitive and fast responsive colorimetric and off-on fluorescent reversible

chemosensors for Hg2+: preparation of a fluorescent thin film sensor," *Dalton Trans.*, vol. 40, 12578-12583 (2011).
[16] Tharmaraj, V. and Pitchumani, K., "An acyclic, dansyl based colorimetric and fluorescent chemosensor for Hg(II) via twisted intramolecular charge transfer (TICT)," *Anal. Chim. Acta*, vol. 751, 171-175 (2012).
[17] Tian, M. Z., Liu, L. B., Li, Y. J., Hu, R. F., Liu, T. F., Liu, H. B., Wang, S., and Li, Y. L., "An unusual OFF-ON fluorescence sensor for detecting mercury ions in aqueous media and living cells," *Chem. Commun.*, vol. 50, 2055-2057 (2014).
[18] Wang, X. Y., Zhao, J. J., Guo, C. X., Pei, M. S., and Zhang, G. Y., "Simple hydrazide-based fluorescent sensors for highly sensitive and selective optical signaling of Cu2+ and Hg2+ in aqueous solution," *Sens. Actuator B-Chem.*, vol. 193, 157-165 (2014).
[19] Yang, R., Guo, X. F., Wang, W., Zhang, Y., and Jia, L. H., "Highly Selective and Sensitive Chemosensor for Hg2+ Based on the Naphthalimide Fluorophore," *J. Fluores.*, vol. 22, 1065-1071 (2012).
[20] Zhang, X. B., Guo, C. C., Li, Z. Z., Shen, G. L., and Yu, R. Q., "An optical fiber chemical sensor for mercury ions based on a porphyrin dimer," *Anal. Chem.*, vol. 74, 821-825 (2002).
[21] Bontidean, L., Mortari, A., Leth, S., Brown, N. L., Karlson, U., Larsen, M. M., Vangronsveld, J., Corbisier, P., and Csoregi, E., "Biosensors for detection of mercury in contaminated soils," *Environ. Pollut.*, vol. 131, 255-262 (2004).
[22] Ivask, A., Virta, M., and Kahru, A., "Construction and use of specific luminescent recombinant bacterial sensors for the assessment of bioavailable fraction of cadmium, zinc, mercury and chromium in the soil," *Soil Biol. Biochem.*, vol. 34, 1439-1447 (2002).
[23] Grattan, K. T. V. and B.T.Meggitt, "Chemical and environmental sensing," in *Optical Fiber Sensor Technology*, vol. 4: Kluwer Academic Publishers, 1999.
[24] Dai, H. J., Liu, F., Gao, Q. Q., Fu, T. H., and Kou, X. M., "A highly selective fluorescent sensor for mercury ion (II) based on azathia-crown ether possessing a dansyl moiety," *Luminescence*, vol. 26, 523-530 (2011).
[25] Nguyen, T. H., Venugopalan, T., Sun, T., and Grattan, K. T. V., "Intrinsic Fiber Optic pH Sensor for Measurement of pH Values in the Range of 0.5-6," *IEEE Sens. J.*, vol. 16, 881-887 (2015).