Biosensors and Bioelectronics 101 (2018) 135–145

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Urinary p-cresol diagnosis using nanocomposite of ZnO/MoS_2 and molecular imprinted polymer on optical fiber based lossy mode resonance sensor

Sruthi P. Usha, Banshi D. Gupta*

Department of Physics, Indian Institute of Technology Delhi, New Delhi 110016, India

ARTICLE INFO

Keywords:
P-cresol
Artificial urine
Optical fiber sensor
Lossy mode resonance
Molecular imprinting
Zinc oxide/ molybdenum sulphide nanocomposite

ABSTRACT

A lossy mode resonance (LMR) based sensor for urinary p-cresol testing on optical fiber substrate is developed. The sensor probe fabrication includes dip coating of nanocomposite layer of zinc oxide and molybdenum sulphide (ZnO/MoS_2) over unclad core of optical fiber as the transducer layer followed by the layer of molecular imprinted polymer (MIP) as the recognition medium. The addition of molybdenum sulphide in the transducer layer increases the absorption of light in the medium which enhances the LMR properties of zinc oxide thereby increasing the conductivity and hence the sensitivity of the sensor. The sensor probe is characterized for p-cresol concentration range from 0 μM (reference sample) to 1000 μM in artificially prepared urine. Optimizations of various probe fabrication parameters are carried to bring out the sensor's optimal performance with a sensitivity of 11.86 nm/μM and 28 nM as the limit of detection (LOD). A two-order improvement in LOD is obtained as compared to the recently reported p-cresol sensor. The proposed sensor possesses a response time of 15 s which is 8 times better than that reported in the literature utilizing electrochemical method. Its response time is also better than the p-cresol sensor currently available in the market for the medical field. Thus, with a fast response, significant stability and repeatability, the proposed sensor holds practical implementation possibilities in the medical field. Further, the realization of sensor probe over optical fiber substrate adds remote sensing and online monitoring feasibilities.

1. Introduction

Natural processes such as decomposition of organic matter, combustion of wood and coal, and production of industrial sewage result in the formation of phenols and its perpetual contamination to water bodies and soil (Del Fino and Dube, 1976). Of these, the most commonly formed and highly toxic phenolic compound is 4-methyl phenol, also termed as p-cresol. Thus, the production rate of p-cresol in environment is considerably high and it reaches the body through many external ways viz. water, air and food. Food items such as tea leaves, oil, tomatoes and tap water possess various types of cresols. The use of p-cresol as a flavouring agent in food items and precursor in traditional medicines results in its increased level in human body. The higher toxicity of p-cresol in human body is due to its hydrophobic behaviour and biotransformation to active conjugated compounds (quinone methide) or in the generation of free radicals (Michalowicz and Duda, 2007). These intermediate compounds interact with cells and tissues in the body resulting in cellular depletion (Bruce et al., 1987). In human body, p-cresol is generated in varying amount as an end product of protein breakdown or by microbial action in the intestines, in addition to its external addition from the environment. The protein stack results in enhanced nutritional absorption by the body as well its excretion mainly through urine. This additional nutrient absorption occurs with the help of colon in the large intestine, where the fermentation process takes place. The concentration of colonial bacteria has been reported to be around 10^{11} to 10^{12} cells/gram which carries out the further metabolic activities and energy production from phenolics such as p-cresol (Hamer et al., 2012). The fermentation process generates p-cresol and its metabolites which are, generally, electrophilic in nature that bind with the macromolecules (enzymes, proteins and DNA) resulting in further toxicity and damage (Ketteler, 2006; Michalowicz and Duda, 2007). This is because the protein binding nature prevents them from getting eliminated from the human body even by dialysis causing kidney failure, cardiovascular diseases, liver damage, high toxicity and finally death (Ketteler, 2006).

In human beings, the presence of p-cresol is generally detected from urine and faeces (Birkett et al., 1995). Various schemes and methods have been reported in the literature for the detection of p-cresol which include mainly enzymatic schemed approach using methods of electrochemical viz. amperometric and potentiometric, optical viz.

* Corresponding author.
E-mail address: bdgupta@physics.iitd.ac.in (B.D. Gupta).

http://dx.doi.org/10.1016/j.bios.2017.10.029
Received 17 August 2017; Received in revised form 10 October 2017; Accepted 13 October 2017
Available online 16 October 2017

spectroscopy, surface plasmon resonance (SPR), and fluorescence, liquid chromatography and gas chromatography (Chriswell et al., 1975; Birkett et al., 1995; De Smet et al., 1998; Li et al., 2006; Kovács et al., 2008; Hasani and Moloudi, 2008; Harper et al., 2009; Singh et al., 2013). These methods possess limitations of expertise monitoring, time consumption, complicated methodology, expensive, cumbersome, low sensitivity and selectivity due to electrode fouling, limited operating period, intensity fluctuations and instability of recognition elements such as enzymes and antibodies affecting sensor response (Li et al., 2006; Singh et al., 2013). The enzyme used in general for p-cresol detection is tyrosinase (Li et al., 2006; Singh et al., 2013), which itself under bacterial fermentation can produce p-cresol (Ketteler, 2006). The reaction of p-cresol with tyrosinase may result in the production of free radicals which again increase the toxicity. Thus, in the case of biosensor probes designed using enzymatic approach for medical applications, the instability of enzyme can be a serious issue. These limitations can be overcome by using the bio-detection scheme of molecular imprinting. The molecular imprinting technique creates highly selective memory encampments called as imprints for the analyte to be detected (Gupta et al., 2016; Shrivastav et al., 2016). The technique involves the preparation of a suitable medium by the polymerization of a monomer in a solvent in the presence of an initiator, cross linker and the template molecule (analyte to be detected). The polymeric medium thus obtained is termed as non-imprinted polymer (NIP) in which the functional groups of template molecules are bound with the functional groups of the polymer. The imprints are then created with the help of a leaching agent that can break the bond between the functional groups of template molecule and polymer. The imprints in the polymeric medium have complementary size, shape and arrangement as that of the analyte. This results in unique re-binding feasibility between the functional groups of polymers and the analyte of interest, since the imprints are created by using the template molecules which are analyte themselves. Hence, molecular imprinted polymer (MIP) ensures selective recognition in sensing. A schematic that briefly illustrates the sensing mechanism using molecular imprinted polymer is shown in Fig. 1(a). These characteristics make molecular imprinted polymer an optimal biorecognition component for sensor design.

The combinational approach of molecular imprinted polymer as biorecognition layer with lossy mode resonance (LMR) as transducer mechanism has been recently introduced by our group (Usha et al., 2017a). Integration of lossy mode resonance with molecular imprinted polymer on optical fiber substrate provides highly sensitive and selective biosensor probe. The usage of optical fiber as substrate holds additional advantages of online monitoring and remote sensing feasibilities (Gupta et al., 2016). Lossy mode resonance is a competing transducer technique to SPR in the field of sensing (Del Villar et al., 2012; Usha et al., 2015). Even though both techniques work on the basis of change in the refractive index of the surrounding medium, the availability of large number of materials supporting lossy mode resonance and the easy realization of resonance using both transverse electric (TE) and transverse magnetic (TM) polarized light make the lossy mode resonance based sensors more in demand these days (Del Villar et al., 2017). Fiber optic LMR based sensor probe can be realized using Kretschmann configuration in which LMR supporting material is coated over the unclad core of the optical fiber followed by the recognition layer (Del Villar et al., 2012). Among the materials supporting LMR such as polymers, semiconductor metal oxides (SMOs) and organometallics, semiconductor metal oxides hold the advantage of higher optical transparency in the visible region, characteristic charge carrier mobility, mechanical flexibility and compatibility with organic and inorganic compounds that make the sensor design simple (Yu et al., 2016). Hence, semiconductor metal oxide has been chosen in the proposed study to realize LMR on optical fiber substrate. The positive real part of dielectric constant of semiconductor metal oxide results in the rearrangement of guided modes in that region and the modes start propagating along the semiconductor metal oxide. The modes that are lost from the guiding core to the semiconductor metal oxide layer are termed as lossy modes. When light impinges on semiconductor metal oxide layer, the mobility of charge carrier changes, which changes the conductivity of the semiconductor metal oxide. As the conductivity changes, the dielectric constant and absorption vary which, in turn, alter the propagation constant of the mode. The change also depends on the dielectric constant of the recognition layer surrounding the semiconductor metal oxide (Batchman and McWright, 1982). Lossy mode resonance occurs when the coupling between the guided mode in the core of optical fiber and the lossy mode in semiconductor metal oxide layer takes place. The coupling occurs due to the overlapping of modal fields and the phase matching between the two (Del Villar et al., 2017). The interaction of the analyte surrounding the recognition layer changes its dielectric constant which results in a change in the condition of resonance. The wavelength at which the resonance occurs, due to the maximum transfer of light from the guided mode to the lossy mode, is termed as the resonance wavelength or peak absorbance wavelength which corresponds to a dip or peak in the transmission or absorption spectrum. This is the basic principle of the operation of a fiber optic LMR based sensor.

In this study, an LMR based fiber optic biosensor for the detection of p-cresol in artificial urine is reported. The sensor is fabricated by coating a layer of nanocomposite of zinc oxide (ZnO) and molybdenum sulphide (MoS_2) on the unclad core of the optical fiber followed by the optimized layer of molecular imprinted polymer. Zinc oxide has been chosen as the semiconductor metal oxide material because of biocompatibility, multiple capability as matrix and carrier, non-toxicity, non-specific adsorption, compatibility with the molecular imprinted polymer, stability and inertness (Özgür et al., 2005; Usha et al., 2016, 2017b). Molybdenum sulphide is a low cost, highly stable material with high carrier mobility and a narrow direct band gap of 1.3–1.8 eV. The addition of MoS_2 in ZnO to form the nanocomposite increases the absorption of light in the semiconductor metal oxide transducer layer (Tian et al., 2016) which increases the mobility of the charge carriers, conductivity and the refractive index of the layer and hence the transducer properties. In other words, the presence of molybdenum sulphide in the nanocomposite changes the dielectric constant and hence the refractive index causing an increment in the sensitivity. The sensor operates in the concentration range 0 μM–1000 μM incorporating the range of conjugated and unconjugated p-cresol in human body.

2. Experimental

2.1. Preparation of ZnO/MoS_2 nanocomposite

Zinc oxide/molybdenum sulphide nanocomposite was prepared in a hydrothermal way. First, the molybdenum sulphide was prepared separately (Zhang et al., 2016). A solution of 2 g of sodium molybdate dihydrate and 2.4 g of thioacetamide was prepared in 160 ml of Millipore water and the mixture was stirred continuously for 1 h. To the mixture, 1.2 g of oxalic acid was added and stirred for another one hour, followed by hydrothermal treatment for 24 h at 150 °C to prepare molybdenum sulphide. To synthesize nanocomposite, 2.1 g of zinc acetate dihydrate and 1.68 g of sodium hydroxide were added to the prepared molybdenum sulphide, and stirred continuously for 1 h. The suspension was hydrothermally treated for 20 h at 100 °C. The prepared nanocomposite of molybdenum sulphide and zinc oxide was kept at 4 °C when not in use. The details of all the chemicals used in the study are provided in S1.

2.2. Preparation of molecular imprinted polymer using free radical-water bath polymerization

To prepare the p-cresol (template) imprinted polymer, p-cresol of 2 mM concentration was dissolved in 18 ml of acetonitrile (porogen).