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Detection of Zn^{2+} Ions Using a Novel Chemosensor Based on Coumarin Schiff-base Derivatives by Electrochemical and Fluorescence Spectroscopy

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Abstract :

We report on the dual detection of zinc ions (Zn^{2+}), using a novel coumarin-based fluorescent chemosensor 7-(diethylamino)-3-((2-hydroxy-4-methylphenylimino) methyl)-2H-chromen-2-one (DHMMC), by using electrochemical and fluorescence based spectroscopy techniques. An efficient electrochemical sensor which consists of gold (Au) interdigitated electrodes (IDE) on a glass substrate was used to perform electrical impedance spectroscopy (EIS) for quantitative detection of Zn^{2+}. Picomolar level detection of Zn^{2+} was made possible by EIS in the presence of DHMMC. Selective detection of Zn^{2+} for concentrations as low as 1 µM was facilitated by fluorescence titration spectroscopy, based on the bathochromatic shift of the different optical signals produced in the emission spectra. The EIS and fluorescence based response were analyzed and is presented in this work.

Key words: dual detection, zinc, fluorescent chemosensor, electrochemical, interdigitated electrodes.

Introduction

Zinc (Zn), the second most abundant heavy metal ion in the human body, is an element that is very critical to life due its role in gene transcription [1], metalloenzyme functions [2], synaptic neurotransmission [3] and mediating neuronal excitotoxicity [4]. Zn, a constituent of various proteins and enzymes, is also essential for the development and growth of both flora and fauna. However, modern industrial development has caused elevated concentrations of heavy metals, including Zn, which leads to toxicity of soil and inhibits plant and animal growth. Thus, there is a need for rapid response, effective detection and reliable identification of trace levels of zinc ions (Zn^{2+}).

There has been significant interest in the design and synthesis of fluorescent chemosensors for the detection of physiologically important ions and molecules [5] and monitoring harmful pollutants in the environment [6, 7]. Although several fluorescent based chemosensors for Zn^{2+} have been developed, they have shortcomings for practical applications such as sensitivity toward other metal ions, susceptibility to pH and difficult syntheses methods [8-11]. It is therefore necessary to develop new chemosensors for Zn^{2+} with high selectivity and sensitivity at physiological pH ranges. Coumarin-based fluorescent chemosensors have received increasing interest in recent years [8, 10] by virtue of their low toxicity, excellent photophysical properties and ease of modification. Direct interaction with the carbonyl group of coumarin is frequently exploited as a good model for chemosensor design, because the spectroscopic response is fast and efficient in the presence of other metals ions.

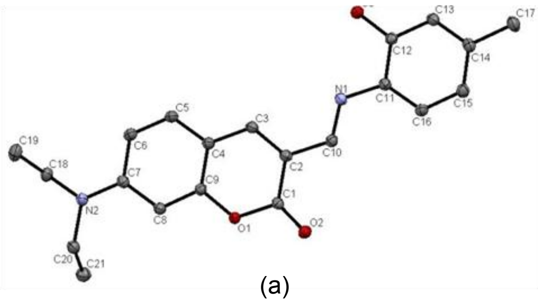
Research has also shown the use of direct electrochemical methods along with electrical impedance spectroscopy (EIS) for the detection of toxic heavy metals [11, 12]. Sensing devices that integrate the dual use of fluorescent and electrochemical detection techniques reduces the possibility of false positives. Therefore the novel approach of using rapid, efficient, portable and miniaturized dual detection sensing systems is crucial to the advancement of opto-electrical sensor technology. The high sensitivity of EIS based electrochemical sensing devices [13] along with research on the integration of these sensors into hand-held portable devices for in-situ detection of heavy metals is important for applications in the medical and environmental agencies.

In this work, we report on the design and synthesis of a new coumarin based chemosensor (7-(diethylamino)-3-((2-hydroxy-4-methyl phenyl imino) methyl)-2H-chromen-2-one (DHMMC)) for the sensitive and selective detection of Zn^{2+} via a chelation-enhanced fluorescence mechanism. In addition, an EIS based efficient sensor device, fabricated using photolithography technique, which employs gold (Au) interdigitated electrodes (IDE) on a glass substrate is used for the electrochemical detection of Zn^{2+}. The EIS and fluorescence based response of the electrochemical sensor towards Zn^{2+} using the coumarin based DHMMC chemosensor is demonstrated.

Experimental

Chemosensor Synthesis: The DHMMC chemosensor was synthesized in good yield by using 4-(diethylamino)-2-hydroxybenzaldehyde as a starting material. 7-diethylaminocoumarin-3-aldehyde (DACA) was then reacted in ethanol at reflux with an equimolar amount of the corresponding ligand to afford the DHMMC compounds in very high yields. The structure of the synthesized DHMMC is shown in Fig. 1a.

Electrochemical Sensor Fabrication: The design and fabrication process of the electrochemical sensor device used in this work has been comprehensively described in a previously published study [14]. The sensor was fabricated on 4 inch round glass wafers via photolithographic technique. This device had overall dimensions of 2 cm × 1 cm × 0.05 cm with 0.1 µm thick Au IDE's that are 5 µm wide and 4995 µm long with 5 µm electrode spacing (Fig. 1b).



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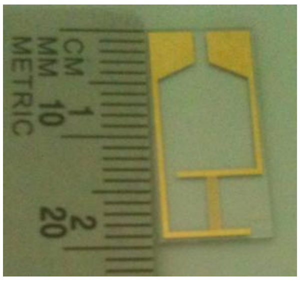

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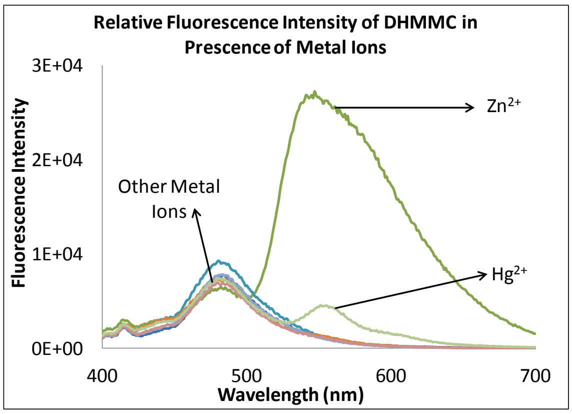
Fig. 1. (a) X-ray crystal structure of DHMMC and (b) Photolithographically fabricated electrochemical sensor device.

Fluorescence Spectroscopy: Fluorescence titration spectroscopy for varying concentrations of Zn^{2+} (0 to 30 µm) in DHMMC was performed Spectrometric measurements were also taken for 3 µM solution of DHMMC in CH_3CN, with different metal nitrates and chlorides (Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Hg^{2+} and Zn^{2+}) as the source of metal ions.

Electrical impedance spectroscopy: The sensor was rinsed with acetone and then blow dried with pressurized air before use. All measurements were conducted in room temperature. Initially, 10 µL of the DHMMC was placed on the sensor to establish a reference signal following which it was washed with acetone and dried in a stream of pressurized air. Then 10 µL of varying concentrations of Zn^{2+} in DHMMC (100 pM, 100 nM, 1 µM and 1 mM) were pipetted, in individual tests, onto the sensor. The impedance measurements were performed using an Agilent E4980A precision LCR meter, connected to the sensor via small outline integrated circuit (SOIC) test clips, at frequency ranges between 20 Hz to 2 KHz, with a 1 mV voltage excitation. Post processing of the measured impedance data was made possible through a custom built data acquisition and analysis LabVIEW program. The sensor was cleaned with acetone and dried in a stream of pressurized air, at the end of each test.

Results and Discussion

Fluorescence: DHMMC showed very weak emission changes when coordinating with some metal ions (Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}) and showed a small emission enhancement with Hg^{2+} (Fig. 2a). By contrast, the addition of Zn^{2+} to solutions of DHMMC resulted in a drastic fluorescence emission change and bathochromic shift. The fluorescence peak for DHMMC at 475 nm red shifted to 550 nm in the presence of Zn^{2+} with a remarkable fluorescence enhancement. The fluorescent quantum yield of the coumarin derivative, DHMMC, increases about 100 fold due to inhibition of the C=N isomerization process upon Zn^{2+} binding at the NOO site. The fluorescent enhancement of DHMMC with Zn^{2+} showed a peak at 550 nm and a peak at 556 nm with Hg^{2+} indicating a red shift of 6 nm from Zn^{2+}. All these observations indicate that DHMMC has high sensitivity and selectivity towards Zn^{2+} over other metal ions tested. The selectivity of DHMMC for Zn can be explained, in part, due to fact that the close-shelled orbitals of Zn do not provide a nonradiative pathway for the excited state electron or energy transfer occurring within the associated complex [15].



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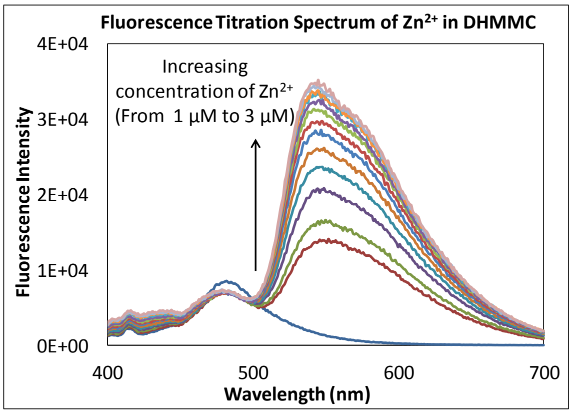
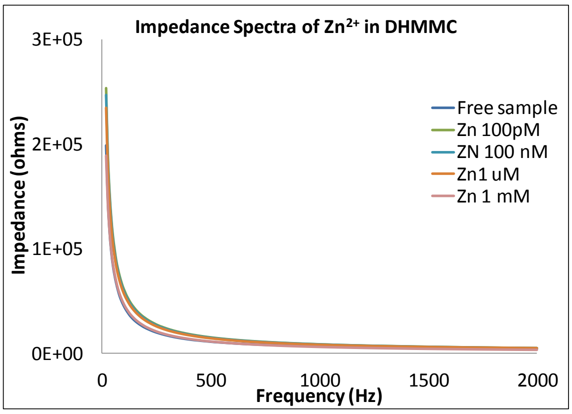


(b)

Fig. 2. (a) Relative fluorescence intensity of DHMMC in the presence of various metal ions (excitation 370nm) (b) Fluorescence changes of DHMMC with [Zn^{2+}] (0 - 30 µM) (excitation 370nm).

The fluorescent titration spectrum shows the fluorescence "turn on" characteristic of the DHMMC due to Zn^{2+} (Fig. 2b). To evaluate the detection limit of Zn^{2+} by the chromophore in solution, the fluorescence changes were measured by increasing the amounts of Zn^{2+}. The fluorescence intensity of DHMMC increased almost tenfold in the presence of 3 µM of Zn^{2+}, indicating a concentration level of detection (LOD) of 1 µM.

Electrochemical Impedance Spectroscopy: Figure 3a shows the impedance response of the electrochemical sensor towards varying concentrations of Zn^{2+} (100 pM to 100 nM to1 µM to 1 mM) in the presence of DHMMC. A distinguishable and better signal to noise ratio was observed at lower frequencies ranging from 100 Hz to 400 Hz. As an example, for measurements at operating frequency of 200 Hz, the impedance response decreased from 34 kΩ to 32 kΩ to 31 kΩ to 25 kΩ as concentrations of Zn^{2+} increased from 100 pM to 100 nM to1 µM to 1 mM, respectively. This response showed that the impedance percentage change achieved with respect to the



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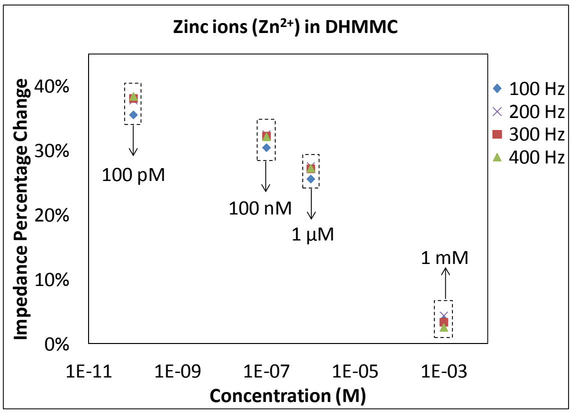

(b)

Fig. 3. (a) Sensor response towards different concentrations of Zn^{2+} in DHMMC, at applied potential of 1 mV and (b) Impedance percentage change at 100 Hz, 200 Hz, 300 Hz and 400 Hz.

DHMMC at 200 Hz were 38 %, 33 %, 28 % and 4 % as the concentration of Zn^{2+} was varied from 100 pM to 100 nM to1 µM to 1 mM, respectively (Fig. 3b).

The change in measured impedance response of the electrochemical sensor is due to the change in charge transfer dynamics between the metal electrodes and varying concentrations of the Zn^{2+} [16-18]. The impedance responses displayed the capability of the sensor device to detect Zn^{2+} concentrations as low as 100 pM and the ability of the sensor to distinguish among a wide range (micro, nano and pico level concentrations) of Zn^{2+}. It is worth noting that the approved level of zinc by the National Academy of Sciences – National Research Council. Food and Nutrition Board (NAS-NRC) is 40 mM [19].

Conclusion

In this work, a new chromogenic chemosensor, DHMMC, based on coumarin Schiff-base derivatives were developed for fluorescence and EIS based detection of Zn^{2+}. DHMMC showed a "turn on" fluorescent response as the emission red-shifted from 475 nm to 550 nm. The high selectivity of DHMMC for Zn^{2+} is evidenced by its exceptional fluorescence enhancement compared to various metal ions. Quantitative detection of Zn^{2+} was also made possible by using a highly sensitive electrochemical sensor device. The response of the electrochemical sensor device towards Zn^{2+} in DHMMC displayed an impedance percentage change of 38 % for concentrations as low as 100 pM when compared to free samples of DHMMC at 200 Hz. The integration of this novel approach of using both fluorescence and EIS techniques into an intelligent dual opto-electrical sensing system for the selective and sensitive detection of a wide variety of biochemicals is a major focus of our future research work.

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