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Quantum dots based platform for application to fish freshness biosensor

K. Kamil Reza[a,1], Manish Kumar Singh[a,b,1], Surendra K. Yadav[a,b,1], Jay Singh[c], Ved Varun Agrawal[a], B.D. Malhotra[d,*]

[a] Department of Science and Technology Centre on Biomolecular Electronics, Biomedical Instrumentation Section, National Physical Laboratory, Dr. K.S. Krishnan Marg, New Delhi 110 012, India
[b] Department of Physics, Motilal Nehru National Institute of Technology, Allahabad 211 004, India
[c] Department of BIN Fusion Technology and Department of Polymer-Nano Science and Technology, Chonbuk National University, Jeonju, Jeonbuk 561-756, South Korea
[d] Department of Biotechnology, Delhi Technological University, Shahbad Daulatpur, Delhi 110 042, India

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ABSTRACT

Laser ablated tin oxide quantum dots (SnO_2 QDs ~1–5 nm) uniformly dispersed in a colloidal solution have been electrophoretically deposited onto indium–tin-oxide (ITO) glass for immobilization of xanthine oxidase (XO_x) for fabrication of fish freshness biosensor. The results of electrochemical response studies conducted on XO_x/SnO_2 QDs/ITO bio-electrode reveal higher sensitivity (0.5148 μA/μM cm^2), lower K_m value (0.022 μM), faster response time (10 s), and wide linear range of 1–400 μM with regression coefficient as 0.999, higher charge transfer rate constant 1.63 s^{-1}.

1. Introduction

The fish production in the present-day world is estimated to have risen from 147 million tonnes in 2010 to 152 million tonnes in 2011 due to increased consumption. As a part of food safety and food security, there is an urgent need for availability of sensitive and cost-effective methods that can be utilized for estimation of fish freshness. In this context, xanthine liberation arising as a result of metabolic function [1] is considered to be an important marker that can be used to indicate the freshness of fish at an early stage. Besides this, abnormal presence of xanthine in a human may lead to a variety of diseases like gout, xanthineuria and kidney failure, due to its accumulation in blood vessels [2].

Quantum dots (QDs) also known as the artificial atoms have recently attracted much interest because of their unique electrical and optical properties arising due to quantum confinement of the energy levels resulting in enhanced charge transport phenomenon [3,4]. The majority of analytes like enzymes, nucleic acids, antigens are known to have specific binding sites that may perhaps be coupled with the nano-sized compatible quantum dots [5]. It has been reported that electrochemical properties of these tiny nanocrystals arise because of their large surface-to-volume ratio, high surface reaction activity and better charge transfer capability [6–11]. The high-performance liquid chromatography (HPLC) and high performance capillary electrophoresis (HPCE) are commonly used analytical methods for detection and quantification of xanthine. These methods are time-consuming, expensive and are not user friendly. Compared to these, electrochemical biosensors are known to be simpler, rapid, sensitive and require no complex sample preparation.

The tin oxide (SnO_2) nanostructures have recently been reported to be biocompatible [12], provide stable physiological environment and are cost-effective. These SnO_2 nanostructures can be utilized for many applications including electrode materials [13], optoelectronic devices [14], sensors [15–17] and as biological labels [18]. The laser ablation in liquid (LAL) has recently been shown to be an interesting technique for fabrication of quantum dots [19]. We report results of studies relating to the fabrication of laser ablated SnO_2 QDs thin film onto indium tin oxide (ITO) via electrophoretic deposition (EPD) technique for application to fish freshness biosensor.

2. Experimental

2.1. Material and methods

Xanthine oxidase (EC1.1.3.22, from microbial source) has been obtained from Sigma, Germany. Tin oxide quantum dots have

* Corresponding author. Tel.: +91 11 27294668; fax: +91 11 27871023.
E-mail address: bansi.malhotra@gmail.com (B.D. Malhotra).
[1] These authors have equally contributed.

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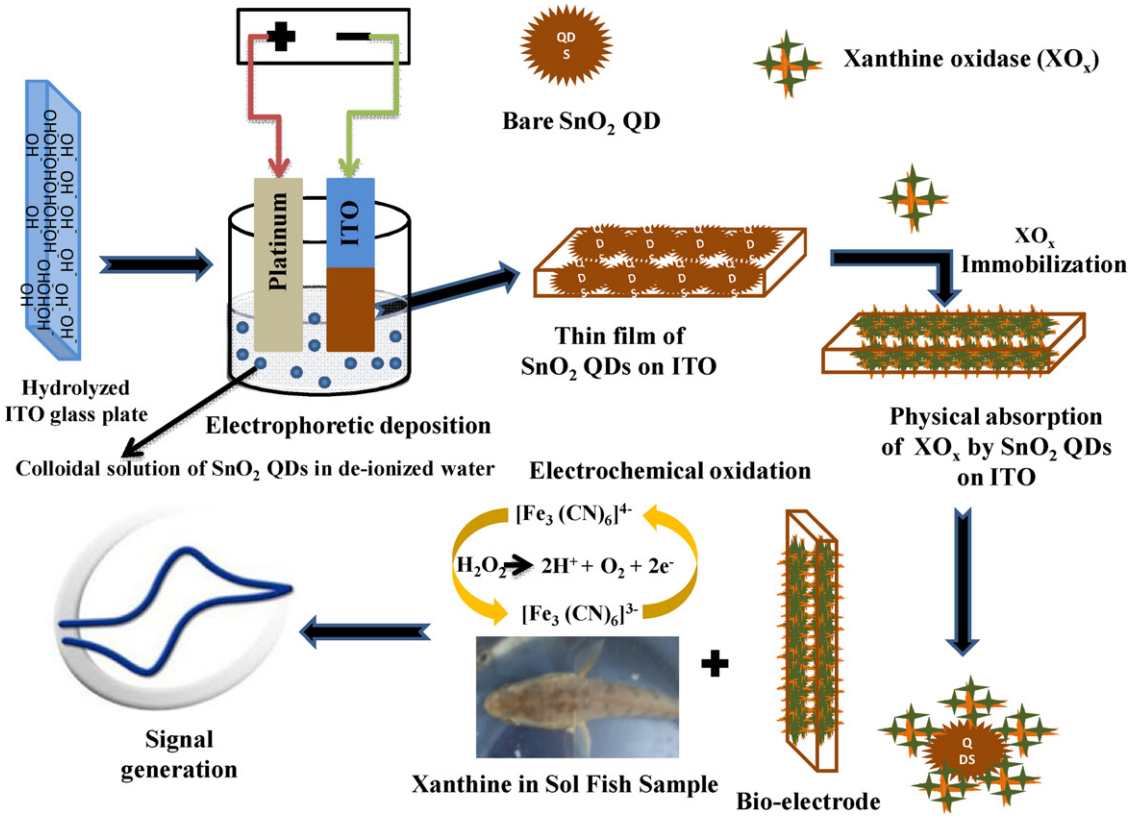

Scheme 1. Schematic illustration for the fabrication of SnO_2 quantum dots based Xanthine Biosensor.

been prepared from tin pellets of 99.9% purity, purchased from (Specpure Johnson Mathey, UK). The xanthine, ascorbic acid, lactic acid, perchloric acid have been purchased from Fine Chemicals New Delhi, India. All other reagents are of analytical grade and have been used as received without any further purification. All aqueous solutions have been prepared in double distilled water. Phosphate buffer solution (PBS) is prepared from Na_2HPO_4 and NaH_2PO_4 reagents.

2.2. Mechanism of SnO_2 quantum dots formation

Tin pellet of 99.9% purity is placed at the bottom of glass vessel containing 20 mL of double deionised water. The pellet is ablated for 60 min by focused beam of 1064 nm of pulsed Nd:YAG (Spectra Physics, Quanta Ray, USA) laser operating at 35 mJ/pulse energy with 10 Hz repetition rate and 10 ns pulse width [19]. A laser pulse interacts with tin immersed in double distilled water and a plasma plume is generated at the liquid–solid interface due to the absorption of the laser pulse. The confined plasma creates a shock wave that results in higher pressure and increase in plasma temperature (6000 K). As a consequence, the ejection of ions, atoms, and clusters from the target may react with liquid molecules at the interfacial region between plasma and liquid at a liquid–solid interface [20]. The transient reactions and rapid quenching result in clusters encounter and interact with the solvent, inducing the following chemical reaction:

$$\mathrm{Sn(clusters)} + 4\mathrm{H_2O} \rightarrow \mathrm{Sn(OH)_4} + 2\mathrm{H_2} \quad (1)$$

$$\mathrm{Sn(OH)_4} \xrightarrow{\text{high (pressure/temperature)}} \mathrm{SnO_2} + 2\mathrm{H_2O} \quad (2)$$

The local high temperature and pressure in the LAL process provide appropriate conditions for the formation of SnO_2. Thus obtained solution of colloidal nanoparticles is collected for characterization and preparation of thin film by electrophoretic deposition technique.

2.3. Bioelectrode fabrication

The proposed mechanism for preparation of XO_x/SnO_2 QDs/ITO bio-electrode and immobilization of XO_x onto this electrode are shown in Scheme 1. ITO (indium tin oxide) coated glass sheets are cut into small pieces (0.5 cm × 2 cm) and immersed in a solution of H_2O_2:NH_3:H_2O in the ratio of 1:1:5 (v/v) and are then kept in an oven for about 1 h at 80 °C. Further the hydrolysed ITO plates are washed with de-ionized water (DW) and dried at room temperature. For the deposition of SnO_2 QDs film, it is dispersed in autoclaved DW with ratio 1:1 and sonicated for about 30 min for proper dispersion of SnO_2 QDs solution prior to EPD. For 0.25 cm^2 area of the electrode, the optimized EPD potential and time are found to be 25 mV and 30 s, respectively. Multiple samples are prepared for reliability studies. Fresh solution of XO_x (0.2 unit/ml) is prepared in PBS (50 mM, pH 7.0) and is uniformly spread (10 μL) onto the desired SnO_2 QDs/ITO electrode. The XO_x/SnO_2 QDs/ITO bio-electrode is stored in a humid chamber for 12 h at room temperature. The bio-electrode XO_x/SnO_2 QDs/ITO thus fabricated is washed thoroughly with PB (50 mM, pH 7.0) containing 0.9% NaCl to remove any unbound enzyme and stored at 4 °C.

2.4. Instruments

The surface topographies of SnO_2 QDs/ITO electrode and XO_x/SnO_2 QDs/ITO bio-electrode have been investigated using atomic force microscopy (AFM) (VEECO, New York, USA) in the non-contact mode. Morphological observations have been carried