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Short communication

Pearl shaped highly sensitive Mn_3O_4 nanocomposite interface for biosensor applications

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ABSTRACT

An electrochemical biosensor based on manganese oxide (Mn_3O_4) and chitosan (Cn) nanocomposite has been fabricated for fish freshness detection. The electrophoretic deposition of Mn_3O_4 nanoparticles (15–20 nm) with Cn has changed their morphological arrangement leading to pearl shaped of Mn_3O_4–Cn nanocomposite on indium tin oxide substrate. Size and morphology of nanocomposite have been confirmed by high resolution transmission electron microscopy (HRTEM), X-ray diffraction (XRD) and scanning electron microscopy (SEM). The results of electrochemical response reveal that this improved sensor has widest detection range of xanthine concentration from 1 to 500 μM and excellent sensitivity of 1.46 μA μM^{-1} cm^{-2}. The fabricated XO_x/Mn_3O_4–Cn/ITO biosensor can detect as low as 1.31 μM of xanthine and lower K_m value of 0.018 μM confirming its superior affinity towards the nanocomposite film.

1. Introduction

The growing demand for fish in the international market has risen considerably in the last decade as large section of human populations living depend on fish industry (Pulvenis et al., 2010). Fish meat freshness is of great importance to food industries for the quality control of fish products. Therefore efforts are going on for estimation of fish freshness by detection of xanthine whose concentrations keep on increasing after the death of fish as a result of metabolic functions. Moreover, xanthine being precursor of uric acid in human body has a clinical significance for kidney related diseases (Berry and Hare, 2003). Thus an efficient device for xanthine detection and quantification is required immediately for clinical analysis as well as fish freshness determination in industry. Xanthine oxidase (XO_x) has been implicated as a key oxidative enzyme by electrochemists for estimation of xanthine (Shan et al., 2009a; Shan et al., 2009b). In this regard, electrochemical detection of xanthine based on nanostructures has been quite successfully reported in literatures (Zhang et al., 2012). Electrochemical sensing has been widely acknowledged as an affordable, rapid, stable, simple and practical technique for a potential miniaturization of devices (Reza et al., 2014, Ali et al., 2014).

In this context, immobilization of xanthine oxidase onto a desired matrix including nanostructured metal oxide (Reza et al., 2013) is considered very important for their high electron mobility, larger surface area for absorption, low detection limit and chemically stable. Apart from that, nano-structured manganese oxide (Mn_3O_4) has shown excellent properties like efficient catalytic activity, high carrier mobility, good biocompatibility, chemical stability and excellent electrochemical properties (Gao et al., 2011). Chitosan (Cn) is a natural cationic biopolymer that has attracted much interest owing to its interesting properties such as inexpensive, stable thin film formation ability and excellent compatibility with biomolecules (Liu et al., 2006). Further, the presence of amino and hydroxyl groups in Cn facilitates in immobilization of enzymes for biosensor application.

Moreover, Cn metal oxide composites are being explored extensively for its excellent sensitivity, high absorption ability, strong enzyme affinity and good stability of the electrodes for clinical investigation of analytes (Kaushik et al., 2009). A very little work based on these materials has been reported in the literature for xanthine sensing (Devi et al., 2012a, 2012b). A combination of Cn and an excellent electrode material like Mn_3O_4 is yet to be attempted for xanthine sensing. We report a fish freshness electrochemical biosensor based on XO_x which was immobilized onto the electrophoretically deposited nanocomposite electrode.

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2. Experimental

2.1. Reagents and apparatus

All chemicals of analytical grade were purchased from Sigma-Aldrich, India. Mn_3O_4 and its composite have been characterized by using X-ray diffraction (XRD) (Rikagu), high resolution transmission electron microscope (HRTEM, Tecnai G20-stwin), scanning electron microscope (SEM, LEO-440), and Fourier transform infrared spectroscopy (FTIR, PerkinElmer). Electrochemial experiments have been conducted on an Autolab Potentiostat/Galvanostat (Eco Chemie, Netherlands) in phosphate buffer saline (PBS) (50 mM, pH 7.0, 0.9% NaCl) containing 5 mM $[Fe(CN)_6]^{3-/4-}$.

2.2. Fabrication of sensor

Colloidal solution of Mn_3O_4 nanoparticles was synthesized using manganese chloride as the precursor. 100 mM of $MnCl_2$ and 200 mM of NaOH are separately dissolved in 100 ml distilled water and ethanol. Nano-Mn_3O_4 was synthesized by the co-precipitation method by using cetyltrimethylammonium bromide (0.3 mM) as a surfactant (Kavas et al., 2010; Chen et al., 2005). The formed precipitate was washed with de-ionized water and ethanol 10 times and dried at 80 °C for 24 h, and then calcined at 400 °C for 3 h. Further, 20 mg Mn_3O_4 is dispersed in 5 ml of Cn solution prepared by dissolving 1 g Cn powder into 100 ml of 0.1 M acetic acid by constant stirring at room temperature. Then 10 ml solution is used for electrophoretic deposition at 5 V for 100 s onto ITO (area = 0.25 cm^2) glass substrate. Fresh solution of XO_x (0.2 unit/ml) is prepared in PB (50 mM, pH 7.0) and is uniformly spread (10 μL) onto the desired Mn_3O_4–Cn/ITO electrode. The fabricated XO_x/Mn_3O_4–Cn/ITO bioelectrode is stored in a humid chamber for 12 h at room temperature. The bioelectrode was kept at 4 °C for further use. The proposed mechanism for preparation of XO_x/Mn_3O_4–Cn/ITO bioelectrode and immobilization of XO_x onto this electrode is shown in Scheme 1.

3. Results and discussion

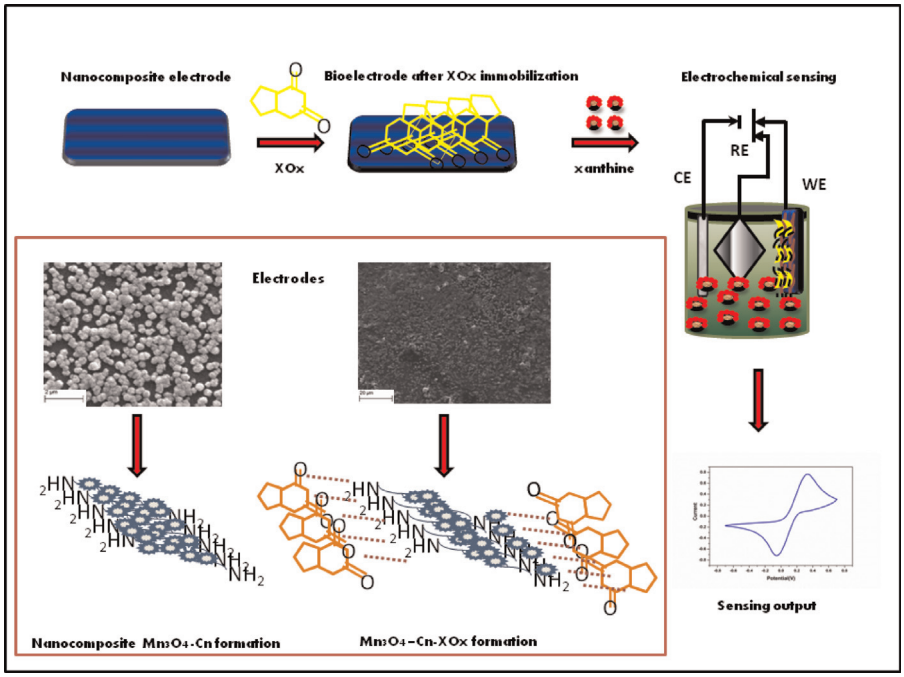
3.1. Characterization of biosensor

HRTEM images [Fig. 1(i) and (ii)] show the presence of Mn_3O_4 nanoparticles. The image shows agglomeration due to inter-particle interactions among the nanoparticles. The nanoparticle size range is about 15–20 nm. The inset images of Fig. 1(i) reveal the crystalline nature of the Mn_3O_4 nanoparticles with lattice fringes corresponding to the (211) and (103) planes (JCPDS file 24-0734) respectively. The '*d*' values of lattice fringes belonging to (211) and (103) planes are 0.250 nm and 0.277 nm, respectively. SAED pattern has been shown in the inset of Fig. 1(ii).

SEM [Fig. 1(iii) and (iv)] studies are carried out in order to examine the electrode surface. Mn_3O_4–Cn/ITO electrode reveals formation of spherical nanocomposite structure on to the surface of ITO in Fig. 1(iii). Plenty of white granules (pearl shaped) of Mn_3O_4–Cn nanocomposite were distributed on the ITO substrate. The surface morphology of nanocomposite electrode behaves as an ordered and oriented active surface area providing a friendly microenvironment for XO_x immobilization. The SEM morphology [Fig. 1(iv)] of enzyme immobilized on Mn_3O_4–Cn/ITO electrode shows a well arranged gel like network, which confirms strong enzyme binding. More SEM images are shown in Supplementary data.

The XRD patterns of the synthesized samples are shown in Supplementary Fig. S1(a). The presence of major peaks of Mn_3O_4 nanoparticles with high intensity confirms the high degree of crystallinity as well as purity of the sample. Almost all the diffraction peaks indexed in Fig. S1(a) have been found to be well matched to the tetragonal structure of Mn_3O_4 (JCPDS file 24-0734).

The FTIR spectra of Mn_3O_4–Cn/ITO and XO_x/Mn_3O_4–Cn/ITO electrodes are shown in Fig. S2(b) of Supplementary file. The Mn_3O_4–Cn/ITO electrode (curve i) shows characteristic peaks of Mn–O at 533 cm^{-1}, 585 cm^{-1} and 866 cm^{-1}. The characteristic peaks at 3424 cm^{-1}, 1561 cm^{-1} and 1263 cm^{-1} are due to O–H, N–H and CH_2 absorption bands of Cn respectively. Further, Mn_3O_4–Cn/ITO electrode shows peaks at 1650 cm^{-1} of N–H band of amines and 1074 cm^{-1} of C–N stretching of amines. Curve (ii)



Scheme 1. Mn_3O_4 nanocomposite based biosensor for fish freshness detection.