Sensibility of polyaniline nanofibers to biomarker of benzene recognized as a carcinogen☆

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Summary   Alarming situation of toxic substances such as benzene and its analogs in the environment and workplaces, making it important to monitor these chemicals and their metabolites in order to evaluate risk hazards and potential problems caused by exposure to toxic compounds. Benzene is omnipresent in usage across industries. International Agency for Research on Cancer (IARC) which is a part of World Health Organization (WHO) has classified Benzene as a human carcinogen. *trans-trans* Muconic acid (*tt*MA) is the most important biomarker of benzene for biomonitoring of its low level exposure. In this paper the sensing ability of polyaniline (PANI) to *tt*MA is investigated for development of cheap, portable and effective electrochemical biosensor. To take this ahead for biosensor device application, successful self-assembling of PANI nanofibers of 40—70 nm range on SS 304 working electrode was achieved. Fourier transform infrared spectroscopy and X-ray diffraction was used to characterize the chemical structure of PANI. Morphology of sample was observed by field emission gun scanning electron microscopy (FEG-SEM). The detection potential of *tt*MA in phosphate buffer solution of pH 5.8 acting as a supporting electrolyte was found to be at 0.26 V by linear sweep voltammetry.

Introduction

Electrochemical biosensor is a versatile tool for determination of various analytes in different fields such as food inspection, clinical diagnosis and environmental monitoring due to its simplicity, faster analysis, portability, low cost and hence it is crucial for early stage screening

of diseases (Prompheta et al., 2015). Various conducting polymers including polyaniline (PANI), polypyrol (PPy) and polyethylenedioxythiophene (PEDOT) are the promising candidates for developing electrochemical biosensors. Among these conducting polymers, PANI is the most promising material due to its excellent electrochemical properties, high environmental stability and low toxicity (Hao et al., 2014). There are various methods of synthesis of PANI such as hard template, soft template, electrospinning, self-assembling electrospraying, electrodeposition, seeding polymerization and interfacial polymerization. Self-assembled nanostructures of PANI on biosensor surface are a well-known, simple and cheap. PANI modified glass, screen printed carbon electrode (SPCE), alumina were used as working electrode for detection of cholesterol (Ruecha et al., 2014), glucose (Kausaite-Minkstimiene et al., 2010), DNA, heavy metals such as lead (Pb) and cadmium (Cd), ammonia, urea and pH determination (Talwara et al., 2014; Lakarda et al., 2012; Song and Choi, 2012). PANI deposited on Stainless Steel (SS) 304 was used as an electrode material for redox supercapacitor and for corrosion inhibition study (Gupta and Miura, 2005; El-Shazly and Al-Turaif, 2012). Due to increasing awareness about toxic substances like benzene and its derivatives in the environment and workplaces, it is becoming important to monitor these chemicals and their metabolites in order to evaluate risk hazards and potential problems caused by exposure to toxic compounds (Chary and Fernandez-Alba, 2012). International Agency for Research on Cancer (IARC) which is a part of World Health Organization (WHO) classified benzene as carcinogen (cancer causing substance) (Weisel, 2010). *tt*MA is considered as an important biomarker of benzene (Boogaard and van Sittert, 1996) to be detected from urine. Commercially available detection techniques of *tt*MA in urine of an individual who has been exposed to benzene are gas chromatography (GC), mass spectrometry (MS) and high precision liquid chromatography (HPLC) (Chiang et al., 2015). But these methods are time consuming and require a large expensive set up and expertise for analyzing urine samples. Hence in this paper, a study was carried out for sensing ability of PANI nanofibers to *tt*MA. This study will initiate the development of cheaper, portable, electrochemical biosensor for detection of *tt*MA from urine sample.

Experimental

Experimentations were carried out to find out the detection potential of *tt*MA present in the phosphate buffer solution and it is discussed as follows.

Materials

Aniline, hydrochloric acid (HCl), ammonium persulfate (APS, $(NH_4)_2S_2O_8$) were all purchased from Merck and *tt*MA was purchased from Sigma Aldrich. All chemicals used were of analytical grade.

PANI nanofibers coated SS 304

PANI nanofibers deposition on SS 304 working electrode involves two independent processes: (1) Preparation of PANI solution and (2) Self-assembling of PANI nanofibers on SS 304.

Preparation of PANI solution. EM PANI salt which is a conductive form of PANI was prepared by mixing aqueous solutions of 0.2 M aniline hydrochloride and 0.25 M ammonium persulfate (APS) at room temperature (Catedral et al., 2004; David et al., 2014).

Self-assembled layer of PANI nanofibers on SS 304. SS 304 electrode was polished with emery paper of grit size 150 to a rough finish. It was then washed with acetone and deionized water to remove emery particles and air dried. SS 304 electrode was then immersed in a beaker containing the above solution and left for self-assembling for 24 h. The total deposition area of SS 304 electrode was 2.02 cm². After 24 h, SS electrode was washed with deionized water and dried at 40 °C for 2 h.

Composition and morphological characterization

FTIR spectroscopy
FTIR spectra of PANI sample have been done using BRUKER TENSOR 27 FTIR spectrometer

Scanning electron microscopy
FEG-SEM analysis was done by Tescan MIRA 3 model with secondary electron (SE) detector at 7.0 kV.

X-ray diffraction (XRD)
XRD was done by X-ray diffractometer- Shimadzu XRD 6100 with scanning rate − 2 degree per minute from 10 to 80° at room temperature. X-ray source used was CuK$_\alpha$, λ = 1.5405° A.

Electrochemical measurement
Electrochemical measurements were carried out with PGSTAT 302N to identify the detection potential at which oxidation of *tt*MA occurs on WE. Current measurements were carried out by LSV in the potential range between 0.0 and 0.50 V. To record LSV, start potential = 0 V, upper vertex potential = 0.5 V, end potential = 0.5 V, step potential = 0.00244 V, scan rate = 0.1 V/s were kept. Electrochemical cell was arranged with PANI deposited on SS 304, saturated calomel electrode (SCE) and platinum (Pt) as working, counter and reference electrode, respectively.

Results and discussion

Chemical structure
Fig. 1 shows the IR spectra of PANI EM salt. Peaks of EM PANI Salt appears at 1557, 1459, 1284, 1235, 1138, and 790 cm⁻¹. Peaks at 1557, 1459 are assigned to C—C ring stretching vibrations. Peak at 1284 and 1235 cm⁻¹ corresponds to N—H bending and symmetric component of C—C (or C—N stretching modes). Bands at 1138 and 790 cm⁻¹ can be characterized the in plane and out of plane C—H bending modes, respectively. In addition to the above peaks, spectrum of EM PANI Salt exhibits new peaks around 3130, 1660, 579 cm⁻¹. The peak at 3130 cm⁻¹ could be attributed to NH_2 stretching mode and 1660 cm⁻¹ band to NH bending vibration while 579 cm⁻¹ is assign to NH_2 wagging. The conducting protonated form band characteristics is observed at 1235 cm⁻¹. C—Cl stretching arises in the region 590−700 cm⁻¹ (Geethalakshmi et al., 2014). XRD analysis of
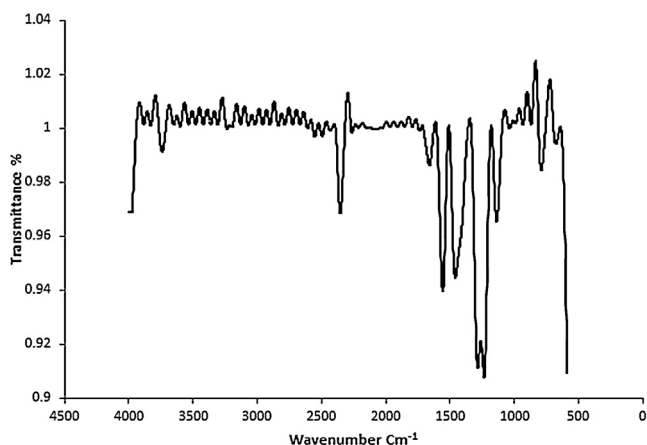
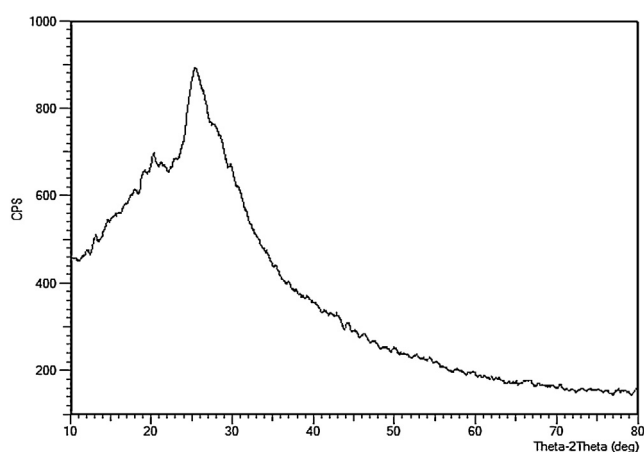**Figure 1**   FTIR spectra of EM PANI salt

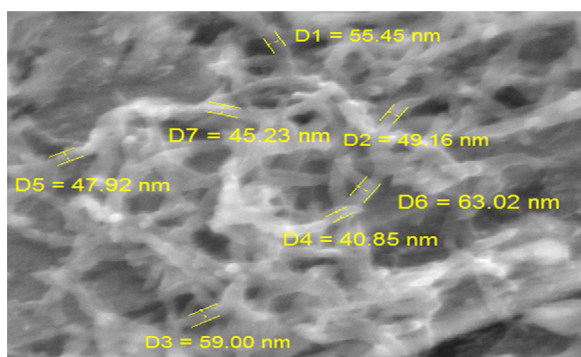**Figure 2**   XRD spectra of EM PANI.

Figure 3   SEM images of nanofibers formed on SS 304 by self-assembling technique.

EM PANI salt shows only a broad amorphous scattering around $2\theta = 25°$ (Basaka et al., 2013) as shown in Fig. 2.

Morphology

SEM micrographs of nanofibers formed by self-assembling are shown in Fig. 3. From SEM image it is observed that nanofibers of diameter ranging from 40 nm to 70 nm are formed.
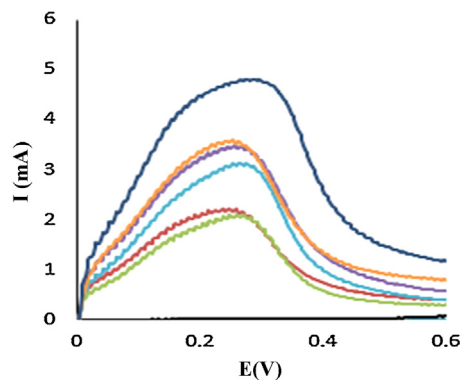

Figure 4   Linear sweep voltammetry showing response profile of 6.4, 9.4, 12.4, 15.4, 18.4, 21.4 mg/L of ttMA in phosphate buffer of pH 5.8.
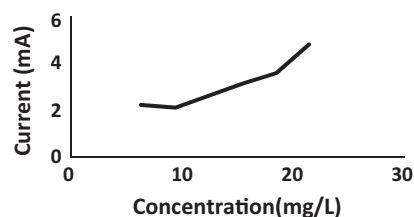

Figure 5   Variation of current due to increase in concentration of ttMA.

ttMA sensing performance

Phosphate buffer solution of pH 5.8 was chosen as supporting electrolyte for detection of ttMA. Sensing behavior of PANI modified SS 304 used as working, SCE as reference and platinum as counter electrode using LSV for varied concentration of ttMA is as shown in Fig. 4. Oxidation current of ttMA exhibits well-defined peaks at 0.26 V. All increase in the peak height for increasing concentration are due to the oxido-reduction reaction occurring at the surface of EM PANI deposited on SS working electrode and ttMA in the solution.

Oxido-reduction reaction occurs due to hydrogen bond formation between carboxylic acid group of ttMA and amino groups of PANI. Here, we can identify the detection voltage of ttMA as 0.26 V. Fig. 5 represents the variation of current for different concentration of ttMA. Minimum ttMA concentration that can be detected by the sensor is 0.8 mg/L

Conclusion

For developing a cheap and portable electrochemical biosensor system for detecting ttMA, the sensing ability of PANI nanofibers on SS 304 electrode to ttMA is carried out. Self-assembling of PANI on SS 304 shows the formation of nanofibers ranging from 40 nm to 70 nm and its chemical composition was verified by FTIR and XRD. Detection voltage of ttMA was found to be 0.26 V by LSV in phosphate buffer solution of pH 5.8 which act as a supporting electrolyte. The increase in current due to increase in the concentration of ttMA was due to oxido-reduction reaction which occurs at the SS 304 coated with PANI. Hydrogen bond formation between carboxylic acid group of ttMA and amino group of EM PANI results in oxido-reduction reaction.

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