www.vbripress.com/aml, DOI: 10.5185/amlett.2016.6163 Published online by the VBRI Press in 2016

# Impedimetric and voltammetry sensing of xanthine using nanocomposites

**Jagriti Narang[1*], Nitesh Malhotra[2], Chaitali Singhal[1], Mitrajeet[1], C. S. Pundir[3]**

[1]*Amity Institute of Nanotechnology, Amity University, Noida, Uttar Pradesh 201313, India*
[2]*Amity Institute of Physiotherapy, Amity University, Noida, Uttar Pradesh 201313, India*
[3]*Maharshi Dayanand University, Rohtak, Haryana 124001, India*

[*]Corresponding author. Tel: (+91) 9811792572; E-mail: jags_biotech@yahoo.co.in

**Received: 13 September 2015, Revised: 25 February 2016 and Accepted: 10 May 2016**

## ABSTRACT

An electrochemical biosensor based on xanthine oxidase (XOx), titanium dioxide nanoparticles and carboxylated multi-walled carbon nanotubes ($TiO_2$/c-MWCNT) nano-composites for sensitive detection of xanthine has been developed. $TiO_2$/MWCNT nano-composites were used as the sensing platform in order to immobilize XOx and magnify the sensor response. FTO electrode was employed to amplify electrochemical signal in the buffer solution. Detailed morphological, electrochemical, structural and optical characterization of XOx/$TiO_2$-NPs/c-MWCNT/FTO electrode was done using XRD, DLS, SEM, EIS, CV and shows quick response time (within 30s), linearity as 0.5- 500 µM, lower detection value of 0.05 micromolar with signal: noise ratio of 3, excellent reproducibility, high selectivity and shelf life of about 8 weeks under refrigerated conditions. The developed biosensor was further used to determine the xanthine levels in the labeo fish samples obtained from market. The accuracy of the developed biosensor was cross-checked by the customary enzymic colorimetric method (99% correlation). Thus, the existing research confirms the development of a highly sensitive, stable and a reliable bio-sensing method to detect the freshness of fish samples. Copyright © 2016 VBRI Press.

**Keywords:** Xanthine; titanium dioxide; xanthine oxidase; multi walled carbon nanotubes; fish freshness.

## Introduction

Xanthine is a type of purine base with the chemical formula 3, 7-dihydro-purine-2, 6 -dione. It is in attendance in the majority of the body tissues and fluids and synthesized from hypoxanthine by xanthine oxidase. Xanthine oxidase is a type of liver enzyme which helps in the metabolism of xanthine. It acts as a catalyst to convert hypoxanthine to xanthine as well as for the conversion of xanthine to uric acid. Excess of xanthine in the body can cause serious ailments like kidney stones and a rare genetic disorder called xanthinuria **[1]**. Hence determination of xanthine in serum/urine is very important in the diagnosis of hyperuricemia, gout, xanthinuria and renal failure **[2]**. Xanthine has also attracted much attention in evaluating the meat freshness, especially in fish. After a fish expires the adenosine triphosphate (ATP) in the fish degrades to form xanthine which keeps on increasing as the storage time increases. Hence it can be said that the larger the amount of xanthine in a fish the lesser is its freshness. Therefore, fish freshness can be determined using xanthine as a marker **[3]**. Xanthine can be determined by enzymatic colorimetric **[4]**, enzymatic fluorimetric, fluorometric fragmentography **[5]**, capillary column gas chromatography **[6]** and HPLC **[7]**. Nonetheless, these methods are burdensome, time intensive and entail costly machinery. XOx has been immobilized onto different supports, for example, poly-pyrrole film **[8]**, self-assembled phospho-lipids membrane **[9]**, theo-phylline coated nylon mesh **[10]**, nafion layer **[11]**, and PVC membrane **[12]**. These supports (membranes/layers) had disadvantages, like meager strength, non-reusability, sluggish electron transport, while a few films are fragile, non-conducting and of pitiable absorption ability **[13]**. Application of aptamers in biosensors has been taken a lot of attention lately because of their high chemical stability **[14]**. However, ribonucleic acid aptamers are vulnerable to get degraded by endogenous ribo-nucleases. Therefore, biosensors based on RNA aptamers are not sufficient enough to apply in biological surroundings. In the time of interminable technological expansion, construction of a compact biosensor for economical and incessant monitoring can effortlessly conquer above precincts **[15]**. Combination of complex chemical, microbiological, and physical processes leads to the loss of freshness and finally spoilage when the most important criterion to meet is quality and to ensure that the food is fresh enough in daily routine basis until reaching the consumers **[16]**. Due to all these drawbacks, nanoparticles based sensors are more preferred. Nanoparticles (NPs) based xanthine biosensors have numerous preferences such as more stability, durability, better sensitivity, accuracy, detection range and faster response time. NPs on the electronically active face of electrode, provides enhanced flow of electrons; thereby connecting the electrolytes in the solution with the sensing platform.

The amalgamation of various nanoparticles leads to the formation of various composites which further enhances the sensing processes. Nanocomposites have magnetized multidisciplinary researches in recent time owing to their

fascinating features for instance increased surface area, increased conductivity and biocompatible microenvironment for biological components **[17]**. They have novel physical and chemical properties that can be incorporated into chemical and biological sensing **[18]**. Semiconductor nanoparticles like titanium dioxide ($TiO_2$) have high surface area, magnetic properties, and low cost, biocompatible, non-toxic, low temperature of processing, high mechanical strength and retention of biological activity which makes them suitable as sensing platform **[19]**. Multi-walled carbon nanotubes are polymers of pure carbon and have distinct characteristics viz. low cost, high yield and are easily produced by well-established processes like Chemical Vapour Deposition (CVD). They also exhibit unique properties like large surface area, fast electron transfer kinetics and biocompatibility **[20, 27, 28]**. Thus, conducting $TiO_2$/CNT platform is dependable for immobilizing bio-molecules, thereby enhancing the efficiency of biosensors. This surface combines the features of CNTs and $TiO_2$ to elevate the electron flow mechanism. This work presents a xanthine biosensor using nanocomposites of titanium dioxide nanoparticles and carboxylated multi-walled carbon nanotubes ($TiO_2$/c-MWCNT) for immobilization of XOx via covalent bonding of XOx and $TiO_2$/c-MWCNT composites on FTO by means of customary coupling agents EDC and NHS. This fabricated technology provides more sensitive and specific method for sensing of Xanthine level in comparison to the previous sensors developed.

## Experimental

*Apparatus and chemicals*

Xanthine oxidase (XOx) (E.C.1.1.3.2) from buttermilk (0.15 U/mg), xanthine extrapure from SRL, Mumbai and c-MWCNT (12 walls, length 15–30 µm, Purity 90%, Metal content: nil) from Intelligent Materials Pvt. Ltd., Panchkula (Haryana) India were used. N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS) were purchased from SRL (Mumbai, India). All other chemicals were of AR grade and all the experiments were carried out in double distilled water.

All the electrochemical measurements were done on a computer-assisted potentiostat (Autolab PGSTAT-10, Eco Chemie, Utrecht, Netherlands), connected to a standard electrochemical unit equipped with XOx/$TiO_2$-NPs/c-M WCNT/FTO as working, Ag/AgCl as reference and Pt wire as counter electrode in PBS (50 mM, pH 7.5, 0.9% NaCl) with 5 mM potassium ferro/ferricyanide in the presence of Xanthine.

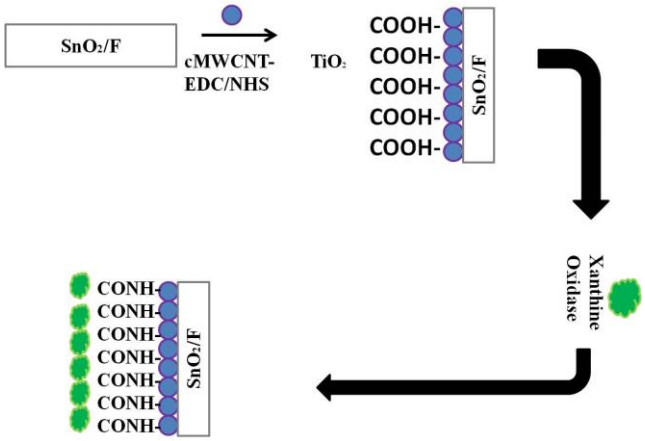
The experimental conditions were controlled with NOVA software. The EIS were performed in 1 mM Fe $(CN)_6^{3-/4}$ with 0.1 M KCl at 0.20 mV $s^{-1}$ (frequency range of 0.01 Hz –50 kHz).

*Fabrication of XOx/$TiO_2$-NPs/c-MWCNT/FTO*

The titanium tetra chloride ($TiCl_4$) was used for the synthesis of $TiO_2$ NPs. $TiO_4$ (50 ml) was slowly added to 200 ml DW in an ice bath. After that, the beaker was kept over magnetic stirrer to make a homogeneous solution for 30 minutes for the synthesis of $TiO_2$ nanoparticles **[21]**.

For the preparation of nanocomposites, the solution containing $TiO_2$ NPs (0.265 g/ 200 ml DW) was prepared with persistent stirring. Further, c-MWCNTs (1.0 mg) were added to it while ultra-sonicating for 10 min. At last, sodium hydrate (0.4 M) was supplemented gradually while maintaining the final pH at 7.0. The solution was subjected to centrifugation technique (5000 × g for 15 min) resulting in black precipitates which were then washed with distilled water. The $TiO_2$/c-MWCNTs were dissolved in methanol and kept in a desiccator at 50 °C for 6 h.

The FTO glass electrodes were diced with a diamond-blade dicing saw, cleaned with HCl: $HNO_3$ (3:1) and air dried. These electrodes were then dipped in to 100 µL of $TiO_2$-NPs/c-MWCNT dispersed solution and were dried in air. This working platform was characterized by SEM and CV at different stages of its construction (**Scheme 1**).




**Scheme 1.** Schematic diagram showing the stepwise fabrication of the modified electrode (XOx/ $TiO_2$-NPs/c-MWCNT/FTO).

The enzyme, XOx was covalently attached onto $TiO_2$-NPs/c-MWCNT nanocomposite layered FTO using NHS-EDC chemistry, as described by Rahman *et al*., (2009)22 with a few changes. Firstly, free –COOH groups of $TiO_2$-NPs/ c-MWCNT composite film were made active by inserting into solution containing EDC and NHS (10 mM) in 0.05 molar PB of pH 7.5 for 6 h. Finally, EDC/NHS treated electrode was incubated in 5 ml of the above prepared PB containing XOx at 4 °C for 3 hours and then washed again with PB. The consequential FTO was dried and stored at 4 °C.

*Characterization*

$TiO_2$ nanoparticles, c-MWCNT and the composites were characterized using XRD, SEM and DLS. Bruker AXS D8 advance X-ray diffractometer driven by Diffrac plus XRD commander software was used for the XRD characterizations. Cu Kα radiation was used with the tube operated at 40kV and 25mA. Surface morphology studies of FTO electrode, $TiO_2$-NPs/c-MWCNT/FTO electrode and XOx $TiO_2$-NPs/c-MWCNT/FTO electrode have been carried out using Zeiss EVO 18 448 scanning electron microscope (SEM) driven by smart SEM software. The samples for SEM were made electroactive by coating with

gold using Quorum SC7620 sputter coater. DLS of $TiO_2$ NPs was performed on Malvern (Nano S 90) Dynamic light scattering instrument. The sample for DLS was made by adding 1 ml of the prepared NPs in a glass cuvette (3 ml) and adding 1.5 ml distilled water to it.

*Voltammetric and impedimetric study*

The voltammetric and impedimetric behavior of the biosensor was studied using potentiostat with XOx/$TiO_2$-NPs/c-MWCNT/FTO as working, Ag/AgCl as reference and Pt wire as counter electrode in PBS as mentioned above. EIS is done to examine the charge transfer processes taking place at electrode/solution interface. CV is done to determine the number of electrons transferred.

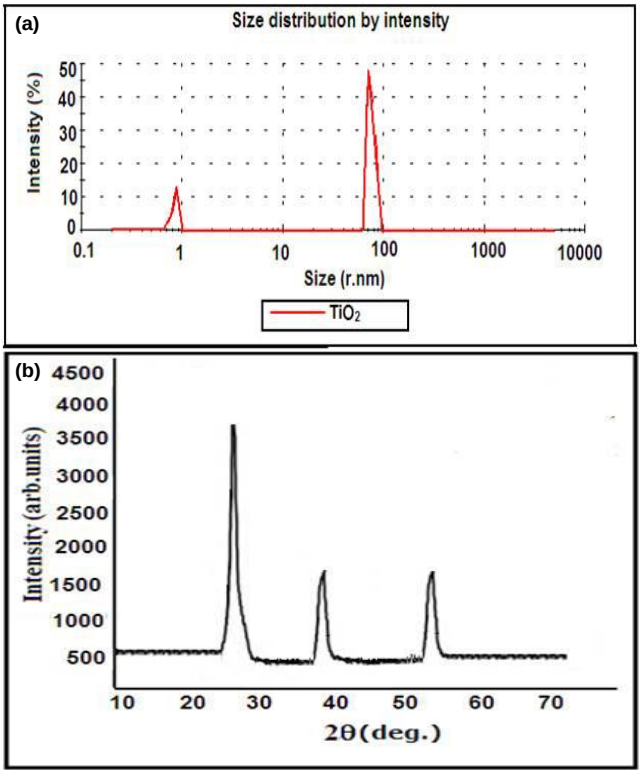



**Fig. 1.** (a) DLS spectra of tin dioxide nanoparticles ($TiO_2$-NPs) and (b) XRD spectra of tin dioxide nanoparticles ($TiO_2$-NPs).

Different optimal conditions are also studied for maximum electrochemical response like pH; temperature and time. For assessment of functioning of biosensor, precision and accuracy parameters were also studied. Fabricated technology was employed to determine xanthine level in Labeo fish purchased from a neighboring marketplace. Fish was chopped and homogenized so that the proteins get precipitated. The denatured samples were subjected to centrifugation at 2000 rpm for 10 min. The obtained supernatant (pH 7.0) was diluted 10 times and divided into two parts. One part was used right away and other was stored at room temperature. To determine xanthine content in fish meat extract, same procedure was used except that xanthine was replaced by the meat extract. The xanthine content was extrapolated from calibration curve plotted between xanthine concentrations vs current (mA).

## Results and discussion

*Evidences of preparation & characterization of working electrode*

DLS measures an average hydrodynamic diameter of particles suspended in an aqueous medium. **Fig. 1(a)** shows DLS of $TiO_2$ NPs, the results of dynamic light scattering showed the size of 85 nm. XRD spectra of $TiO_2$ NPs also match with the literature which confirms the formation of nanoparticles (**Fig. 1(b)**). These results confirmed the formation of nanoparticles.

Different stages of sensing electrode were studied through SEM techniques. The surface morphologies of FTO electrode, $TiO_2$-NPs/c-MWCNT/FTO electrode and XOx/ $TiO_2$-NPs/c-MWCNT/FTO electrode were studied by SEM (**Fig. 2**). Unmodified FTO demonstrated rough surface (**Fig. 2 (a)**), while nanoparticles modified electrode depicts rod and sphere like structure which confirm deposition of nanoparticles and MWCNT (**Fig. 2 (b)**). After bio conjugation of enzyme on electrode, more circular like structures are seen which confirms immobilization of enzyme (**Fig. 2 (c)**).

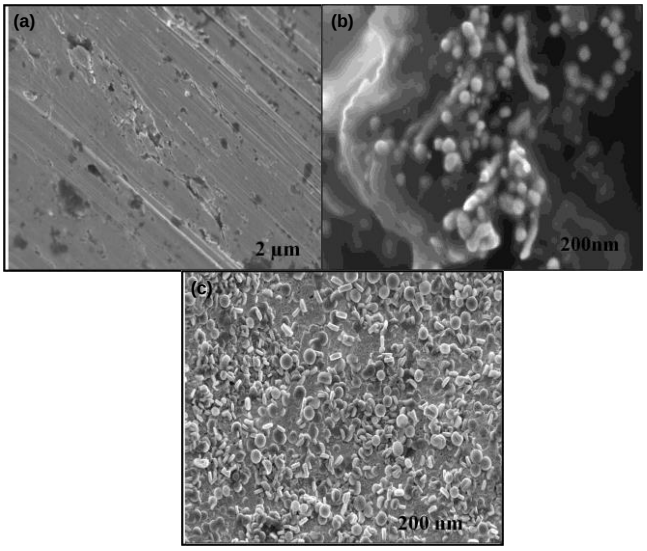



**Fig. 2.** SEM image of (a) bare FTO (b), $TiO_2$-NPs/c-MWCNT/FTO (c) and XOx/ $TiO_2$-NPs/c-MWCNT/FTO.

Electrochemical analysis was done to validate the presence of various sensing materials. CV pattern of different phases of sensing electrode was studied (**Fig. 3(a)**). Unmodified electrode showed less electrochemical sensing (trace a) in scanning potential range of 0.0–0.7 V. MWCNT modified FTO electrode depicts increase in electrochemical signal. The conjugation of nanoparticles to the electrode produces larger amplified electrochemical signal (trace b). After bioconjugation of enzyme on sensing interface the electrochemical signal was promoted further (trace c). It means hybrid nanocomposite provided large surface area and close proximity of enzyme and analyte which helps in increasing the reaction.

Electrochemical impedance spectroscopy (EIS) was also done in order to validate the platform. This technique is very effective for inquiring the surface properties of sensing electrode. Electrochemical impedance spectra were taken at

different phases of FTO (**Fig. 3(b)**). EIS of bare FTO (a) $TiO_2$-NPs/c-MWCNT/FTO (c) and XOx/ $TiO_2$-NPs/c-MWCNT/FTO (b) was performed. The RCT obtained were 1300 Ω and 1000 Ω for hybrid nanocomposite modified electrode (c) and enzyme modified electrode (b) respectively. All results of CV and EIS are matching with each other which proved the formation of sensing electrode. **Fig. 4** describes electrochemical impedometric response of electrode before and after the addition of analyte. Small Rct value (620 Ω) was observed after addition of analyte while Rct value increased to 1100 Ω without addition of substrate showing that the fabricated sensor is specific toward xanthine.

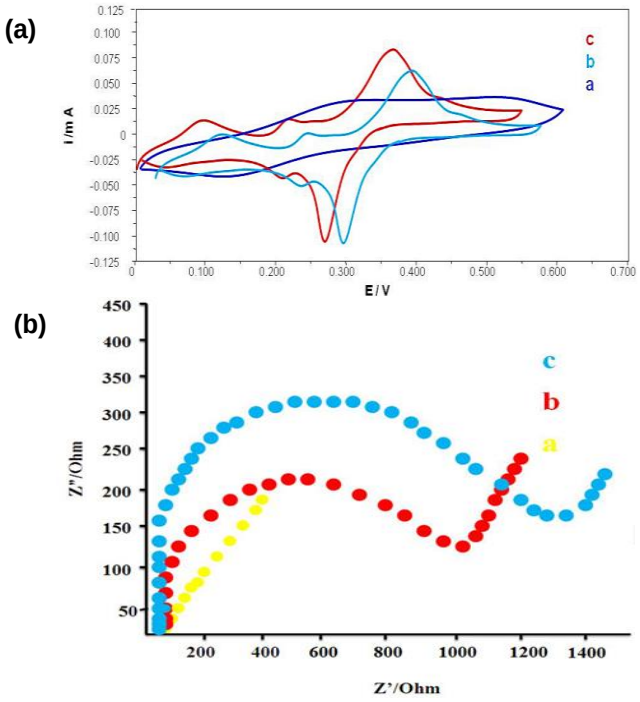



**Fig. 3.** (a) CV pattern of (a) bare FTO (b) $TiO_2$-NPs/c-MWCNT/FTO (c) XOx/ $TiO_2$-NPs/c-MWCNT/FTO in the scanning potential range of -0.1 to +0.1 V $s^{-1}$ at the scan rate 20 mV $s^{-1}$ in 0.1 M phosphate buffer pH 7.5 in the presence of xanthine. (b) EIS of (a) bare FTO (c)$TiO_2$-NPs/c-MWCNT/FTO (b) XOx/ $TiO_2$-NPs/c-MWCNT/FTO containing 1 mM $Fe(CN)_6^{3-/4-}$ with 0.1 M KCl at 0.20 mV $s^{-1}$ in the presence of xanthine. (frequency range of 0.01 Hz –10 kHz).

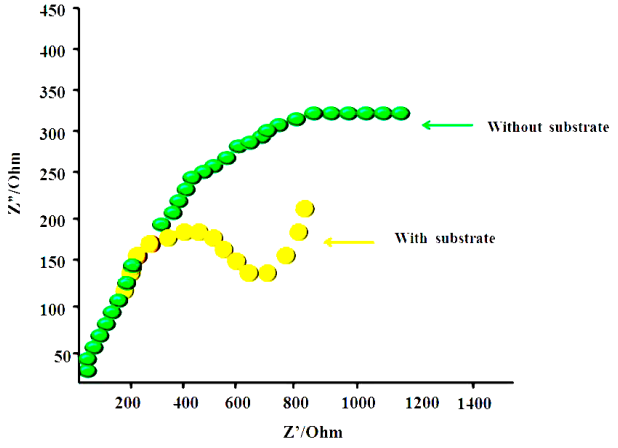



**Fig. 4.** Impedometric response of sensor (a) presence of substrate (b) absence of substrate.

*Impedometric detection of xanthine*

The association between the electron transfer resistance (Rct) and the xanthine concentrations was varied in the range of 0.5 to 500 µM and was studied. Time of incubation was kept 30 s, after 30 s electrochemical signals was produced. Value of Rct decreased with increase in xanthine concentration (**Fig. 5(a)**).

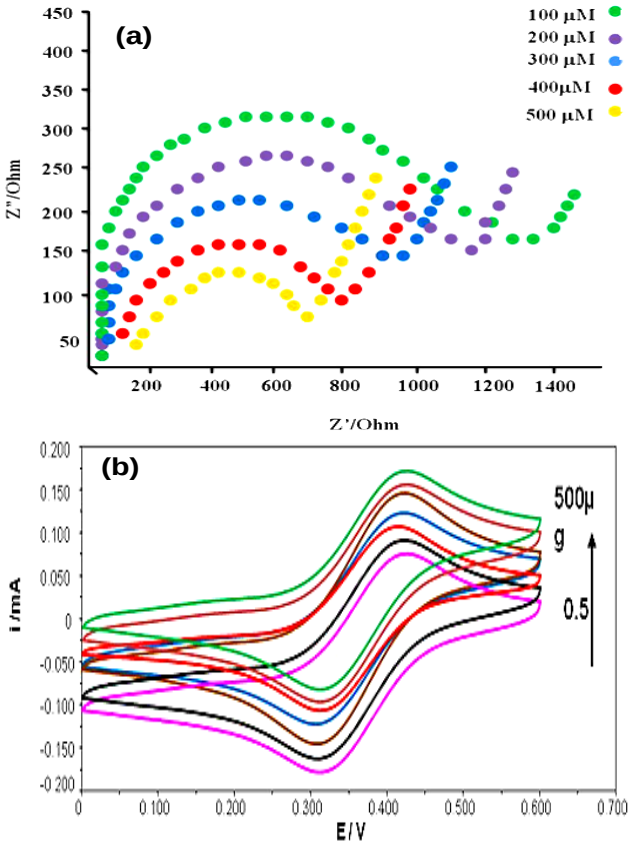



**Fig. 5.** (a) Impedometric response of sensor for xanthine detection (b) CVs of modified electrode using different concentrations of xanthine.

**Table 1.** Interference effect of various compounds on Xa sensor.

| Interferents | Relative response (%) |
|---|---|
| Glucose | 110 |
| Fructose | 100 |
| Ethanol | 100 |
| Ascorbic acid | 140 |
| Citric acid | 100 |
| Lactic acid | 100 |
| Malic acid | 100 |
| Tartaric acid | 100 |
| Alanine | 100 |
| Leucine | 100 |
| Urea | 104 |
| Uric acid | 100 |
| Cholesterol | 85 |
| Pyruate | 100 |

It might be due to the interaction of analyte with the sensing interface which generates electrons. Increase in concentration of xanthine produces more electrons. Electrons are directly correlated with the sensing signal. Sensing signal is inversely related with the RCT. The

reproducibility obtained was 3% after performing the experiments thrice. Calibration graph was also made using CV with rising concentrations of xanthine (0.5- 500 µM) (**Fig. 5(b)**). Increase in concentration of analyte also causes increase in anodic current. Limit of detection was found to be 0.05 µM.

*Optimization & analytical performances of sensor*

Various parameters were studied for Optimization of sensing electrode like pH, temperature, and time and substrate concentration. The performance of the developed sensor was checked at various scanning potentials from 20 to 100 $mVs^{-1}$. The developed sensor performed well at 100 $mVs^{-1}$. The pH from 5.0 to 7.0 was studied. The electrochemical results were best accomplished at 7.0. Temperature effect was also studied between 25 to 60$^{o}$C. 35$^{o}$C was found to be the most suitable temperature. Time effect was also studied in the range of 1 to 8 s. The modified electrode showed maximum response at 2 s. The effects of different serum interferents such as glucose, uric acid, urea and cholesterol are also noted. None had any noteworthy intervention (**Table 1**).

Evaluating parameters were also studied for sensitive detection of analyte. Analytical recoveries of exogenously added xanthine (10 mg/l, 20 mg/l) in fish sample were 98.2% and 97.1% respectively, presenting the consistency of the process. Substrate level in same fish sample was examined five times on a single day (within batch) and again after one week (storage at −20$^{0}$C; between batch). Coefficients of variation for fish sample determination were < 4.1% and < 4.4%. For the accuracy of present technique, level of xanthine in fish sample was examined by enzymic colorimetric method (x) and by the present method (y) (**Fig. 6** and **7**). Both the techniques were comparable with a good correlation (r = 0.99, significant at 1% level).

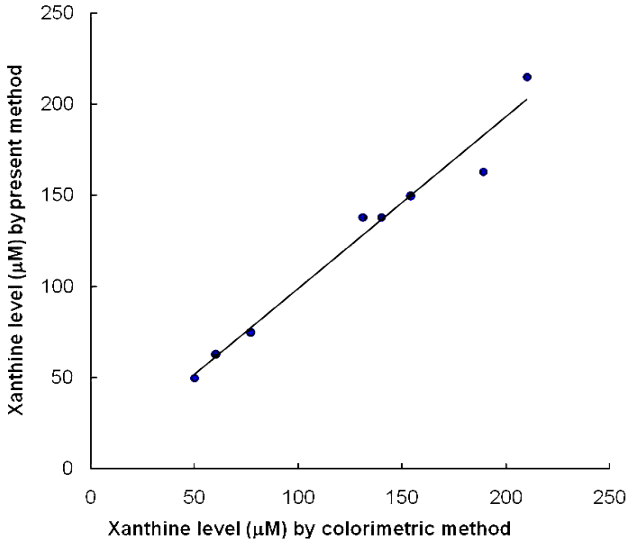



**Fig. 6.** Correlation between Xanthine (µM) determinations by standard enzymic colorimetric method (x) and present amperometric method (y).

Analysis depicted that the sensing platform lost only 40% of the original activity subsequent to 200 uses over 60 days. A comparison of analytical parameters of miscellaneous biosensors for detection of xanthine with the current biosensor is tabulated in **Table 2**.

**Table 2.** Comparison of the present method with other biosensing methods.

| Matrix/method | Enzyme | Response time (s) | Detection limit (µM) | Linearity (µM) | Stability days | Reference |
|---|---|---|---|---|---|---|
| Prussian Blue(PB)+ Polypyrrole (PPy)+ Au-colloid | XOx | - | 1 | 1 - 20 | | [22] |
| Nafion | XOx | <30 | 0.52 | 0.2 -180 | 10 days | [23] |
| Au-NPs/GC | XOx | <5 | 0.1 | 0.1 -100 | 7 days | [24] |
| DWNT | XOx | 150 | 2 | 2 -50 | - | [25] |
| polyvinylferrocenium coated Pt electrode | XOx | - | 0.520 | 1730–1740 | - | [26] |
| titanium dioxide and nanoparticles multiwalled carbon nanotubes | XOx | 30 | 0.5 | 0.5- 500 | 60 | Present |

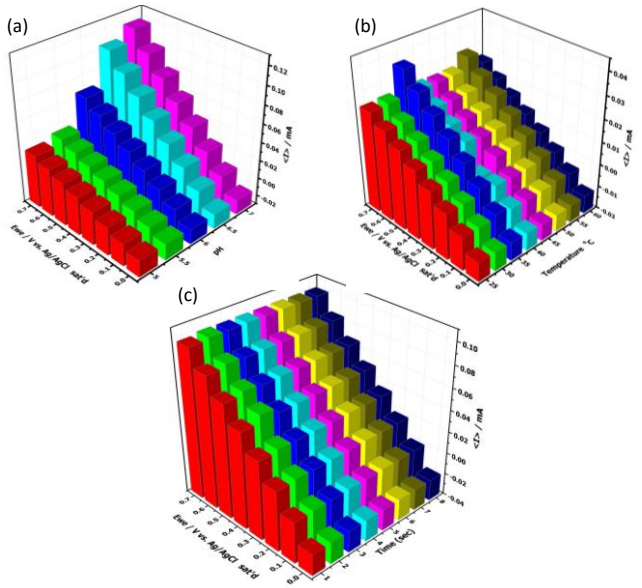



**Fig. 7**. 3D voltammograms obtained at XOx/ $TiO_2$-NPs/c-MWCNT//FTO for different (a) (pH 5, 5.5, 6, 6.5 and 7) (b) Temperature (25, 30, 35, 40, 45, 50, 55, 60 $^{0}$C) (c) Time (1-8 sec).

## Conclusion

Various sensors such as DNA chip can achieve detection sensitivities upto pM and fM; but the cumbersome procedure, high cost and huge time consumption associated with these makes our sensor better than all the other sensors. In our research, XOx/$TiO_2$-MWCNT/FTO electrode has been fabricated for the detection of xanthine level in labeo fish sample. This nanocomposite modified electrode proves to be a promising material for immobilization of XOx, enhancing electron transfer kinetics. Electrochemical results confirmed that the nanocomposite modified electrode showed amplified sensing signal. Moreover, xanthine biosensor exhibits superior biosensing features, viz. linearity as 0.5 to 500 µM, prompt reaction time of 30 s, & specificity. This promising sensing interface can also be utilized for construction of other biosensors.

### Author's contributions

Conceived the plan: Jagriti Narang, Nitesh Malhotra, C.S.Pundir; Performed the experiments: Chaitali Singhal, Mitrajeet; Data analysis: Jagriti Narang Nitesh Malhotra, C.S. Pundir; Wrote the paper: Jagriti Narang. Authors have no competing financial interests.

**Reference**


1. Devi, R.; Narang, J.; Yadav, S.; Pundir, *C.S. J. Anal. Chem*. **2012**, *67*, 273.
2. Narang, J.; Pundir, *C.S. Int J Biol. Macromol*. **2011**, *49*, 707.
3. Shan, D.; Wang, Y.; Xue, H.; *Cosnier, S. Sensor. Actuat. B. Chem*. **2009**, *136*, 510.
4. Berti, G.; Fossati, P.; Tarenghi, G.; Musitelli, C.; Melzid'Eril, G.V. *J. Clin. Chem. Clin. Biochem*. **1988**, *26*, 399.
5. Olojoa, R.O.; Xiab, R.H.; Abramsona, J. *J. Anal. Biochem*. **2005**, *339*, 338.
6. Pagliarussi, R.S.; Freitas, L.A.P.; Bastos, J.K. *J. Sep. Sci*. **2002**, *25*, 371.
7. Kock, R.; Delvoux, B. *J. Clin. Chem. Clin. Biochem*. **1993**, *31*, 303.
8. Arslan, F.; Yasar, A.; Kilic, E. *Art Cells Blood Subst. Biotechnol*. **2006,** *34*, 113.
9. Rehak, M.; Snejdarkova, M.; Otto, M. *Biosens. Bioelectron*. **1994**, *9*, 337.
10. Moody, G.J.; Sanghera, G.S.; Thomas, J.D. *R. Analyst*, **1987**, *112*, 65.
11. Nakatani, H.S.; Santos, L.V.; Pelegrine, C.P.; Gomes, M.; Matsushita, M.; Souza, N.E.; Visentainer, J.V. Am. *J. Biochem. Biotechnol*. **2005**, *1*, 85.
12. Pundir, C.S.; Devi, R.; Narang, J.; Singh, S.; Jyoti, Shewta. *Journal of Food Biochem*. **2012**, *36*, 21.
13. Narang, J.; Minakshi, Bhambi, M.; Pundir, C.S. *Int. J. Biol. Macromol*. **2010**, *47*, 691.
14. McCauley, T.G.; Hamaguchi, N.; Stanton, N. *Anal. Biochem*. **2003**, *319*, 244.
15. M. Dervisevic et al. *Sensors and Actuators B*. **2016**, *225*, 181.
16. Sadeghi, S.; Fooladi, E.; Malekaneh, M. *Anal. Biochem*. **2014**, *464*, 51.
17. Jortner, J.; Rao, C.N.R. *Pure Appl. Chem*. **2002**, *74*, 1491.
18. Rammal, A.; Brisach, F.; Henry, M. C. *R. Chimie*. **2002**, *5*, 59.
19. Narang, J.; Chauhan, N.; Jain, P.; Pundir, C.S. *Int. J. Biol. Macromol*. **2012**, *50*, 672.
20. Shihabi, Z.K.; Hinsdale, M.E.; Bleyer, A.J. *J Chromatogr B Biomed Appl*. **1995**, *669*, 163.
21. Parthasarathi, V. *Paint India*, **2008**, *97*, 104.
22. Rahman, M.M.; Umar, A.; Sawada, K. *Sens. Actuat. B.* **2009**, *137*, 327.
23. Liu, Y.; Lo, N.; Tao, W.; Yao, S. *Electroanal*. **2004**, *16*, 1271.
24. Cubukcu, M.; Timur, S.; Anik, U. Talanta, **2007**, *74*, 434.
25. Anik, U.; Çevik, S. *Microchim Acta*. **2009**, *66*, 209.
26. Baş, S.Z.; Gülceb, H.; Yıldıza, S. *J. Mol. Catal B Enzymatic*, **2011**, *72*, 282.
27. Pundir, C.S.; Devi, R. *Enzyme and Microbial Technology*, **2014**, *57*, 55.
28. Devi, R.; *et al*., *Biochemical Engineering Journal*, **2011**, *58*, 153.

