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Electrochemical biosensor based on REGO/Fe_3O_4 bionanocomposite interface for xanthine detection in fish sample

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ABSTRACT

In this study, xanthine molecules which can serve as an indicator of meat spoilage were determined using a novel and sensitive amperometric xanthine biosensor. Biosensor was developed by preparing a nanocomposite film that was constructed by embedding reduced expanded graphene oxide (REGO) sheets decorated with iron oxide (Fe_3O_4) nanoparticles into poly(glycidyl methacrylate-co-vinylferrocene) (P(GMA-*co*-VFc)) phase, and by covalent immobilization of Xanthine oxidase (XOD) on the surface of P(GMA-co-VFc)/REGO-Fe_3O_4 nanocomposite film. Bio-analytical optimal experimental conditions such as response time, linear range, operation and storage stability, working pH and temperature were studied. Current response of linear range was detected in the range of 2–36 μM with a sensitivity of 0.17 μA/M, response time of ~3 s, and detection limit of 0.17 μM. The resulting bio-nanocomposite xanthine biosensor was subjected to fish real sample testings where 5, 8, 10, 13, 15, and 20 days-old fish samples' xanthine content was measured. The developed biosensor was found to be applicable to real samples as a very reliable fish freshness controlling technique.

1. Introduction

The need of fish freshness tracking that demands on rapid and reliable testing, in acceptable quality is of high importance in food industries to manufacture safe and qualified products. Xanthine is generated from guanine and hypoxanthine being both generated from adenosine triphosphate (ATP). When respiration and biosynthesis of ATP ceases, present nucleotide in muscle undergoes degradation, according to following sequence;

ATP → ADP → AMP → IMP → I → Hx → X → UA

adenosine di phosphate (ADP), adenosine 5′ phosphate (AMP), inosine 5' phosphate (IMP), inosine (I), hypoxanthine (Hx), xanthine (X), and uric acid (UA) (Kassemsarn, Sang, Murray, & Jones, 1963; Nakatani et al., 2005; Pundir & Devi, 2014; Venugopal, 2002), among which IMP is major factor contributing to flavor of fresh fish and its degraded product Hx imparts the bitter taste of fish meat (Mulchandani, Luong, & Male, 1989). On the other hand, xanthine determination in blood sample and tissue is essential for diagnosis of different diseases such as gout, hyperuricemia, xanthinuria, and renal failure (Devi, Thakur, & Pundir, 2011). The most commonly used methods for analysis of xanthine are high pressure liquid chromatography (HPLC) (Kock, Delvoux, & Gresiling, 1993), enzymatic fluorometric assay, fluorometric mass spectrometry fragmentography (Olojoa, Xiab, & Abramsona, 2005), capillary column gas chromatography (Renata, Pagliarussi, Luis, Freitas, & Bastos, 2002), and enzymatic colorimetric assay (Berti, Fossati, Tarenghi, Musitelli, & Melzideril, 1988). Although, the methods provide fruitful results, they suffer from some drawbacks such as being just available in specialized laboratories with very expensive equipments, requiring skilled personnel to operate with it, being not quite suitable for on site measurements, demand of long time for sample preparation, and lack of high sensitivity and specificity (Devi, Batra, Lata, Yadav, & Pundir, 2013) which hardly fulfill contemporary necessities in the time of unceasing technological development, where we can produce small compact devices

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such as biosensors having the potential of easily overcoming these limitations. Amperometric detection of xanthine by biosensors is simpler, cheaper, more sensitive, rapid, and selective requiring no complex sample preparation (Shah, 1996). The basic principle and electrochemical reaction of amperometric xanthine biosensors are based on the reaction below (Devi et al., 2013):

$$\text{Xanthine} + O_2 + H_2O \xrightarrow{XOD} \text{Uric acid} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{V } vs \text{ (Ag/AgCl)}} 2H^+ + O_2 + 2e^-$$

$$2e^- \rightarrow \text{Working electrode}$$

Xanthine Oxidase has been widely used in biosensors' design for fast quantitative analysis of xanthine in real samples (Devi et al., 2013), by immobilizing it onto various supports such as nafion membrane (Nakatani et al., 2005), polypyrrole (PPy) film (Lawal & Adeloju, 2012), polyvinyl chloride (PVC) membrane (Pundir, Devi, Narang, Singh, & Shewta, 2012), self-assembled phospholipids membrane (Rehak, Snejdarkova, & Otto, 1994), cellulose acetate membrane (Basu, Choudhury, & Chakraborty, 2005), silk membrane (Mao, Xu, Xu, & Jin, 2001), and silk fibroin membrane (Mulchandani et al., 1989). However, these supports have several technical drawbacks such as slow electron transfer, poor stability, lack of re-usability, non-conducting and non-elastic nature, fragile and poor absorption ability (Devi et al., 2011), which made requirement for introducing a system modified by electron transfer mediator providing highly desirable lower detection limit and faster electron transfer (Çevik, Şenel, & Abasıyanık, 2010). Ferrocene mediators are widely used to construct mediated amperometric biosensors since they are excellent in electron transfer (Cass et al., 1984 . Rajesh, Pandey, Takashima, & Kaneto, 2005). The essentiality of development of polymeric mediators for applications in biosensors is because it allows the incorporation of reagents (Çevik et al., 2010). Selective xanthine detection can be improved by using mediators, and by facilitating the electron exchange between enzyme active site and electrode (Dodevska, Horozova, & Dimcheva, 2010). Erden, Pekyardımcı, and Kılıç (2012) reported amperometric enzyme electrode for xanthine determination with 1,4 benzoquinone and poly (vinylferrocene) as mediators, Arslan, Yaşar, and Kiliç (2006) reported study on amperometric biosensor for xanthine determination prepared from xanthine oxidase immobilized on polypyrrole film, with ferrocene as a mediator, or Dodevska et al. (2010) reported design of amperometric xanthine biosensor on a graphite transducer patterned with noble metal microparticles. Some widely used redox copolymers trialing the covalent attachment of ferrocene are poly(vinylferrocene-co-hydroxyethyl methacrylate) (Saito & Watanabe, 1998), poly(-glycidyl methacrylate-co-vinylferrocene) (Şenel, Çevik, & Abasıyanık, 2012), acryl amide copolymers (Kuramoto, Shishido, & Nagai, 1994), poly(N-acryloylpyrrolidine-co-vinylferrocene) (Koide & Yokoyama, 1999), ferrocene-containing polythiophen derivatives (Abasıyanık & Şenel, 2010), and multiwalled carbon nanotubes (Kandimalla, Tripathi, & Ju, 2006).

Magnetically functionalization and nanostructured composite have been accepted as novel type alternative materials to create multifunctional magnetic hybrids for specific biosensor applications such as reported by Yang, Zhang, Zhang, and Bai (2014). which synthesized a novel potentiometric glucose biosensor by attaching Fe_3O_4–enzyme–Polypyrrole nanoparticles to the surface of magnetic glassy carbon electrode (MGCE), and Chen et al. which prepared magnetic core–shell Gold–Silica coated Fe_3O_4 nanocomposite by layer-by-layer assembly technique and used this material to fabricate a novel bienzyme glucose biosensor (Chen et al., 2011).

Graphene is attractive material because of its excellent electrical conductivity, high surface area, good mechanical strength, high thermal conductivity and high mobility of charge carriers (Artiles, Rout, & Fisher, 2011; Wang et al., 2009; Wu et al., 2010). The high density of edge-plane defect sites on graphene provides multiple active sites for electron transfer to biospecies. Many researchers have concluded that electrodes made from graphene exhibit significantly more uniform distribution of electrochemically active sites than do those made from graphite (Pumera, 2009). It's entire volume is exposed to the surrounding due to having two dimensional (2D) structure which makes it very efficient in detection of adsorbed molecules (Choi, Lahiri, Seelaboyina, & Kang, 2010) and appropriate platform for electrochemical sensing and biosensing (Pumera, 2009).

In this work, we focused on i) the construction of a novel amperometric xanthine biosensor by a covalent immobilization of XOD, ii) use of incredible properties and abilities of nanotechnology by developing polymeric mediator/graphene oxide/iron oxide nanocomposite, iii) reaching excellent electrochemical properties which will be of high reliability in detection of xanthine, iv) and applicability of biosensor in real sampling of fish freshness testings. Next to successful optimization of biosensor and detailed characterization, we can report excellent application in the detection of xanthine in fish meat, as such applicable in food control system. In addition, Fe_3O_4 magnetic nanoparticles have also attracted an increasing interest in biotechnology and medicine (Cao & Hu, 2006). Due to their properties of having good biocompatibility, strong superparamagneticity, low toxicity, high adsorption ability, and easy preparation procedure, Fe_3O_4 has been widely investigated as electrode modifying material in sensors and biosensors (Cao & Hu, 2006; Cheng et al., 2009). However, to the best of our knowledge, the determination of xanthine using graphene, polymeric mediator and Fe_3O_4 nanocomposite modified electrode has not yet been investigated. The developed biosensing electrode showed rapid electrocatalytic response for xanthine monitoring, and provided a promising platform for prospective biosensor development.

2. Experimental part

2.1. Materials

Xanthine oxidase, xanthine, vinylferrocene (VFc) and Glycidyl methacrylate, Iron (III) nitrate nonahydrate, $Fe(NO_3)_3 \cdot 9H_2O$, iron (II9 chlorur tetrahydrate ($FeCl_2.4H_2O$), sulfuric acid (H_2SO_4 fuming), hydrogen peroxide (30% H_2O_2), potassium permanganate ($KMnO_4$), ammonia solution (28% NH_3), sodium nitrate ($NaNO_3$), hydrazine hydrate ($N_2H_4.H_2O$) and hydrochloric acid (37% HCl) were purchased from Merck and Sigma–Aldrich. All the chemicals employed in the study were analytical grade and used as-received without a purification. The EG was a commercial grade, thermally expanded product (TIMREX® BNB90), kindly provided by TIMCAL (Switzerland). The density, surface area, average particle size (d_{90}) and the oil adsorption number (OAN) values of EG were reported as 2.24 g/cm^3, 28 m^2/g, 85.2 μm and 150 ml/100 mg, respectively by the producer.

2.2. Synthesis

2.2.1. Synthesis of REGO

In the first step, EG was oxidized by the well-known modified Hummers (Hummers & Offeman, 1958) method to prepare EGO. In the second step, EGO powder was dispersed in the deionized water