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Review

Biosensing methods for xanthine determination: A review

Chandra Shekhar Pundir*, Rooma Devi

Department of Biochemistry, M D University, Rohtak 124 001, Haryana, India

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ABSTRACT

Xanthine (3,7-dihydro-purine-2,6-dione) is generated from guanine by guanine deaminase and hypoxanthine by xanthine oxidase (XOD). The determination of xanthine in meat indicates its freshness, while its level in serum/urine provides valuable information about diagnosis and medical management of certain metabolic disorders such as xanthinuria, hyperurecemia, gout and renal failure. Although chromatographic methods such a HPLC, capillary electrophoresis and mass spectrometry are available for quantification of xanthine in biological materials, these suffer from certain limitations such as complexity, time consuming sample preparation and requirement of expensive apparatus and trained persons to operate. Immobilized XOD based biosensors have emerged as simple, rapid, sensitive and economic tools for determination of xanthine in food industries and clinical diagnosis. This review article describes the various immobilization methods of XOD and different matrices used for construction of xanthine biosensors, their classification, analytical performance and applications along with their merits and demerits. The future perspectives for improvement of present xanthine biosensors are also discussed.

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* Corresponding author. Tel.: +91 9416492413.
E-mail addresses: chandraspundir@gmail.com, pundircs@rediffmail.com (C.S. Pundir).

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1. Introduction

Xanthine (3,7-dihydro-purine-2,6-dione) is generated from guanine and hypoxanthine (both generated from ATP degradation) by guanine deaminase and xanthine oxidase (XOD), respectively. Xanthine is subsequently converted to uric acid by XOD, which is excreted through urine.

$$ATP \rightarrow ADP \rightarrow AMP \rightarrow IMP \rightarrow HxR \rightarrow Hx \rightarrow X$$

whereas ATP = adenosine triphosphate, ADP = adenosine diphosphate, AMP = adenosine monophosphate, IMP = inosine monophosphate, HxR = inosine, Hx = hypoxanthine, X = xanthine.

Xanthine is the first indicator of an abnormal purine profile, and can serve as a marker of acute hypoxia stress [1]. Xanthine level in serum increases under several clinical conditions like depressed purine salvage pathway such as Lesch-Nyhan syndrome, however, decreased XOD activity leads to xanthinuria, a genetic disease of xanthine metabolism. Unchecked xanthinuria results into kidney stone formation, urinary tract disease and muscle diseases, due to deposits of xanthine in muscle. Xanthine is excreted rapidly through the kidney, about 10-times faster than uric acid, however, xanthine has low solubility, which can lead to stone formation. Xanthine level is very low in serum, which is about 100-times lower than that of uric acid [2]. The concentration of xanthine in urine is normally extremely low, because it is converted immediately to uric acid, a normal end-product of purine metabolism (Fig. 1).

Hence determination of xanthine in serum/urine is very important in the diagnosis and medical management of hyperuricemia, gout, xanthinuria and renal failure [2].

Xanthine has also attracted much attention in evaluating the meat freshness, specially in fish, as after the death of a fish, ATP gets degraded into xanthine, which increases with storage of fish meat [3]. Freshness of the fish meat is essential in food and pharmaceutical industries for manufacturing of high quality products.

The level of uric acid in normal human urine ranges from 2 to 8 mM [4], while xanthine varies between 41 and 161 μM [5]. However, the quantitative analysis of xanthine in food and clinical samples is a difficult task, due to the matrix complexity and low concentrations of the target compounds. Enzymic colorimetric [6,7] enzymatic fluorimetric, fluorometric mass spectrometry fragmentography [8], high performance liquid chromatography (HPLC) [1,9–11], capillary column gas chromatography [12,13] and Flow injection [14] have been employed for determination of xanthine in biological materials. Although, the methods provided fruitful results, these are cumbersome, require time consuming sample preparation and available only in highly specialized laboratories equipped with very expensive equipments and trained personnel to operate. However, biosensing methods/biosensors overcome these limitations, due to their simplicity, rapidity, specificity, sensitivity, low cost and requirement of relatively economic equipment [4].

2. Biosensors

A biosensor can be defined as an analytic device incorporating a biological sensing element connected to a transducer to convert an observed response into a measurable signal, whose magnitude is directly proportional to the concentration of a that specific chemical or set of chemicals in the samples [15]. Immobilized enzyme based biosensors either consume oxygen e.g. all the oxidases, or produce hydrogen peroxide (excluding oxidases which produce water), or generate (indirectly) the reduced form of β-nicotinamide adenine dinucleotide (phosphate), NAD(P)H, e.g., dehydrogenases, during the course of the oxidation of the substrate of interest [16].

2.1. Basic principle of xanthine biosensors

XOD based electrochemical biosensors have been employed widely for diagnosis and medical management of xanthinuria, muscle disease, gout, liver disorders, kidney stone and heart failure and also measurement of meat freshness in food industries. The electrochemical reactions of these XOD based amperometric xanthine biosensors are summarized below:

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

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

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

When xanthine dehydrogenase (XDH) was applied in place of XOD, the following electrochemical reactions occurred in xanthine biosensors:

$$\text{XDH(ox)} + \text{Xanthine} \rightarrow \text{XDH(red)} + \text{Uric acid}$$

$$\text{Mediator(ox)} + \text{XDH(red)} \rightarrow \text{Mediator(red)} + \text{XDH(ox)}$$

$$\text{Mediator(red)} \text{Mediator(ox)} e + - \rightarrow \text{Working electrode}$$

Xanthine biosensors have been used widely as devices for fast quantitative analysis of xanthine in real samples. In these biosensors, XOD has been immobilized onto various supports such as polypyrrole film [17], nafion membrane [18], self-assembled phospholipids membrane [19], theophylline coated nylon mesh [20,21], cellulose acetate membrane [22], silk membrane [23], polyvinyl chloride (PVC) membrane [24] and silk fibroin membrane [25]. However, these supports (film/membranes) had few drawbacks such as poor stability, reusability and slow electron transfer, while other supports such as membranes were fragile, non-conducting, non-elastic and had poor absorption ability. Recent xanthine biosensors were based on electrochemical hybrid electrodes using Cu(II) hypoxanthine absorptive complex at a hanging mercury drop electrode (HMDE) [26], copper platinum hexachloride/glassy carbon (CuPtCl6/GC) [27], sodium montmrillonite-methyl violate carbon paste [23], Au–colloid polypyrrole layer [28,29], graphite rod [30], carboxylated multwalled nanotubes/polyaniline (c-MWCNT/PANI) [31], zinc-oxide-nanoparticles-plypyrrole (ZnONPs-PPy) [32], platinum nanoparticles (PtNPs) [33] and calcium carbonate nanoparticles $CaCO_3$NPs) [34].

2.2. Methods used for XOD immobilization

The most important step in the development of an enzyme sensor is the attachment of the enzyme onto the surface of the working electrode. This process is governed by various interactions between the enzyme and the electrode material which affects the performance of a biosensor in term of its sensitivity, stability, response time and reproducibility. A variety of methods have been employed for immobilization of XOD, ranging from physical adsorption and entrapment to covalent/chemical bonding for construction of xanthine biosensors as given below.

2.2.1. Physical adsorption

Physical adsorption of XOD is carried out by its simple deposition onto the surface of working electrode and attachment through weak bonds such as Vander Waals forces and electrostatic interactions between the XOD and transducer [32–37].

Merits: It is the simplest and cheap way of immobilization.There is no damage to enzyme as well as no chemical change in the

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