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Review

Biosensing methods for xanthine determination: A review

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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.

Contents

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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

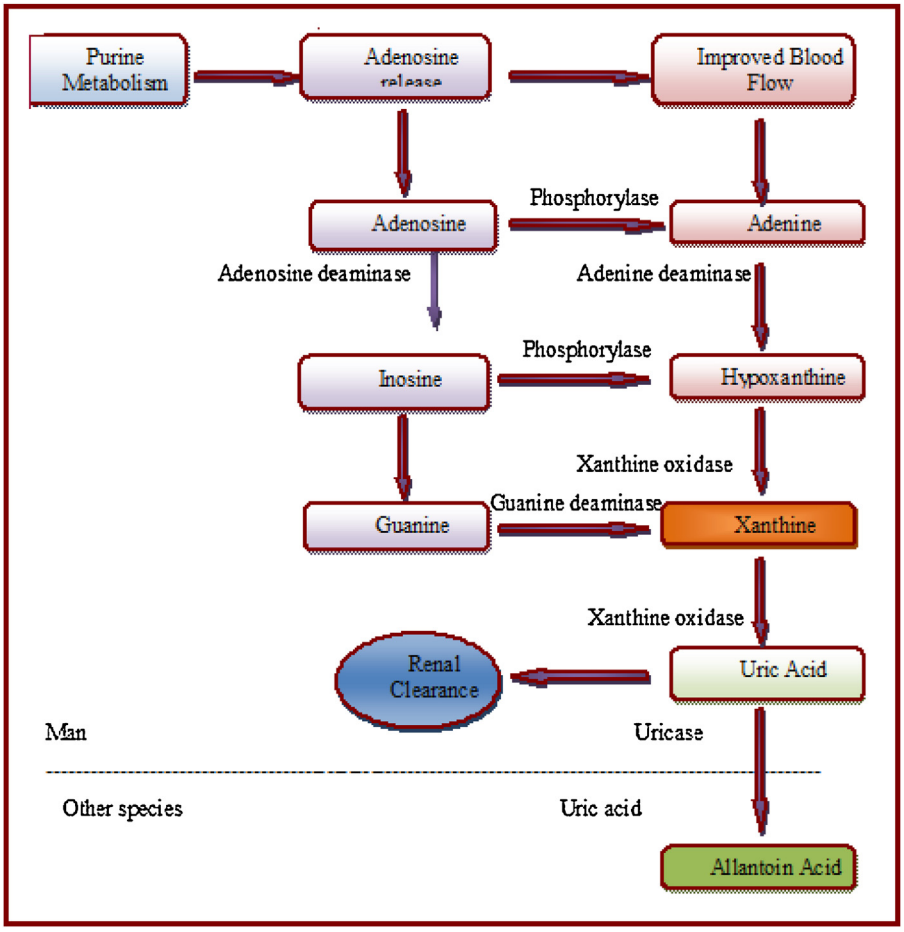

Fig. 1. Scheme of xanthine metabolism in humans and other species.

support. It is also reversible and thus allows regeneration of free enzyme.

Demerits: There are leakage of enzyme, non specific binding, overloading of the enzyme on support, short response time, poor storage stability and sensitivity to changes in pH, temperature and ionic strength.

2.2.2. Entrapment

Entrapment in sol–gel matrices and lattice of a polymer matrix or membrane has also been used for immobilization of XOD onto electrodes in such a way as to retain enzyme, while allowing penetration of substrate.

Merits: It is also simple and cheap way of immobilization which includes one-step procedure at ambient or low temperature. There is no damage to enzyme as well as no chemical change in the support. Hence, it is suitable for a large variety of bio-receptors [38–40].

Demerits: There are leakage of enzyme, non specific, unstable, low reproducibility (85%) and limited pore size and restricted entry of substrate due to diffusion barriers.

2.2.3. Covalent coupling

It is the most widely used procedure for immobilization of enzyme. XOD has been covalently linked onto the surfaces of a transducer through formation of a stable covalent bond between surface groups of XOD and the transducer [31,41,42].

Merits: There are no diffusion barriers, short response time, no enzyme leakage, wide range of choices for selecting carrier material which make the method flexible with specific chemical and physical properties.

Demerits: It is expensive as it requires high amount of enzyme and includes possibility of enzyme denaturation and complicated procedure.

2.2.4. Electropolymerization

The electropolymerization process involves polymerization under the influence of an electric current. Electropolymerization is performed by holding the film of a polymer at a suitable oxidation potential or by using consecutive cyclic voltammetry in a suitable positive scan potential region [43].

Merits: The method has the ability of coating electrodes, which have a small or non-uniform surface, the control of the polymer thickness and the prevention from interferences or electrode fouling. Many polymers behave as mediators.

Demerits: Electropolymerization requires conducting substrates. This limits the types of surfaces which can be electropolymerized.

2.2.5. Cross-linking

This method is based on the formation of covalent bonds between enzyme molecules, through bi- or multifunctional reagent such as glutaraldehyde, leading to three dimensional cross linked aggregates. This procedure has several steps such as the mixing of the biological and chemical components under optimized conditions of pH, temperature and ionic strength followed by a period of incubation, during which the chemical reaction proceeds. Immobilized materials are washed thoroughly for removal of non-bound biological components that would easily interfere in the solution and distort the data [24,44,45].

Merits: Cross-linking is the best method used in conjunction with the other method. It is used mostly as a means of stabilizing adsorbed XOD and also preventing its leakage. It provides very strong immobilization of enzyme.

Demerits: Cross-linking may cause significant changes in the active site of enzymes. Further severe diffusion limitation of cross linked enzyme may lead to significant loss of its activity.

Xanthine biosensors can be classified into two major classes based on their evolution and support materials used.

2.3. Classification of xanthine biosensors on the basis of evolution

There are three 'generations' of xanthine biosensors based on binding of XOD onto transducer:

2.3.1. First generation xanthine biosensor

In the first generation of the biosensor, the biocatalyst is entrapped between or bound to membrane and fixed upon the surface of the transducer. An amperometric xanthine biosensor based on XOD immobilized onto prussian blue (PB)-membrane modified Pt electrode represents first generation biosensor. In this biosensor, H_2O_2, a product of xanthine oxidation by immobilized XOD, was detected at the PB-membrane modified electrode by both electrooxidation and electroreduction. PB-membrane modified Pt electrode was a better electrocatalyst for H_2O_2 oxidation than the bare Pt electrode [46].

2.3.2. Second generation X biosensor

In second generation biosensor, there is covalent immoblization of biologically active component onto the transducer's surface, which eliminates the use of the semi permeable membrane e.g. enzyme-membrane electrodes using XOD in combination with peroxide detection which dominate in the field of laboratory analyzers for diluted samples. Using the same indication principle, extremely fast responding xanthine biosensors have been fabricated by covering thin metal electrodes with a porous enzyme layer. In the second generation biosensors, auxiliary enzymes and/or co-reactants are co-immobilized with the analyte, in order to improve the analytical quality and simplify the performance [22,24,46].

2.3.3. Third generation

The third generation biosensor arise from self-contend nature of the sensor, where the reaction itself causes the response and no product or mediator diffusion is directly involved. The direct binding of biocatalyst to an electronic device transduce and amplifiy the signal. Since neither mediator nor enzyme is added, this design facilitates repeated measurements. Conducting polymers are widely used to make such biosensors [32,47,48].

2.4. Classification of xanthine biosensors based on support material used

2.4.1. Membrane based xanthine biosensors

These biosensors (based on the immobilization of enzyme on suitable matrixes) offer a portable, cheap and rapid method for determination of xanthine. The exotic properties of biocompatible artificial membranes provide the promising matrixes for enzyme immobilization to enhance the sensitivity and selectivity of biosensors. The development of biosensors based on immobilized enzymes has solved several problems such as loss of enzyme (especially if expensive), maintenance of enzyme stability and increased shelf life of the biosensor and reduction in time for the enzymatic response and also offer disposable devices easily applicable in stationary or in continuous flow systems.

Supports used for immobilization of XOD: Nafion membrane [18], self-assembled phospholipids membrane [19], theophylline coated

Table 1
A comparison of analytical characteristics of various xanthine biosensors.

Support for immobilization	Method of immobilization	Optimum pH	Detection limit	Linear range	Response time	Interfering compounds	Storage stability	Application	Reference
Self-assembled biotinylated phospholipid Membrane	Streptavidin coupling	7.5–7.7	0.02 mM	Upto to 1 μM	<1 min	Ascorbic acid	5 days	–	[19]
$CuptCl_6$/GC	Glutaraldehyde coupling	7.6	0.1 μM	0.6 μM–0.2 mM	<15 s	–	7 days (30% Retained)	–	[26]
Graphite powder modified ferrocene + eicosane + PVC	Adsorption	5.5	0.2 μM	Upto 50 μM	–	–	35 days (60% lose)	–	[38]
Modified graphite	Covalently coated with gelatine	8.4	4.5 μM	0–40 μM	2 min	L-ascorbic and uric acid	470 h	–	[41]
Nafion	Glutaraldehyde coupling	7.0	0.52 μM	0.2 μM–0.18 mM	30 s	–	10 days	Fish meat	[18]
Au-NPs/GC	Adsorption	7.5	0.1 μM	0.1 μM–0.1 mM	–	–	7 days (18% Retained)	Fish meat	[36]
ZnO–NPs–PPy	Adsorption	7.0	0.8 μM	0.8–40 μM	5 s	–	100 days (40% lose)	Fish meat	[32]
Double walled carbon nanotube	Adsorption	–	2 μM	2–50 μM	150 s	–	–	Fish sample	[54]
AuPPy	Glutaraldehyde coupling	7.2	0.4 μM	0.4–100 μM	4 s	Ascorbic acid	100 days (40% lose)	Fish, Chicken, Pork, Beef meat	[29]
c-MWCNT/PANI	Covalently	7.0	0.6 μM	0.6–58 μM	4 s	Ascorbic acid	100 days (50% lose)	Fish meat	[31]
Prussian Blue(PB) + Polypyrrole (PPY) + Au–colloid	Adsorption		1 μM	1 μM– 20 μM	–	–	–	–	[35]
Didodecyldimethylammonium bromide (DDAB) + tetrathiafulvalene (TTF)	Adsorption	7.5	0.3 μM	0.4 μM–2.4 μM	<10 s	–	–	–	[37]

Table 2
A comparison of analytical characteristics of biosensors based on xanthine oxidase.

Biosensors	Edet/technique	Analyte/sample	K_M^{app} (mM)	Linear range (μM)	Slope, AM^{-1} (×104)	LOD (μM)	Repeatability R.S.D. (%)	Useful lifetime	Ref
XOD–CPE	0.4 V vs. Ag/AgCl/amp.	Hx, X	0.027	5–130; 5–100	6.1; 5.6	–	–	6 days	[55]
XOD-PPy-PtE	0.60 V vs. SCE/amp.	X	2.8	0.1–1000	1.8	0.1	–	–	[52]
XOD-PMBQ-AuGCE	0.30 V vs. Ag/AgCl/amp	Hx/X	0.040	1–50 (Hx), 1–80 (X)	–	–	–	4 days	[51]
XOD-Au coll-GCE	0.4 V vs. Ag/AgCl/amp	X	–	Up to 30	390	0.2	–	7 days (4 °C)	[28]
XOD–FCAPPy–ITO	0.70 V vs. SCE/amp.	Hx	–	Up to 2000	84.2	–	–	7 days	[57]
XOD-SilK-FiB + CA-PtE	0.65 vs. Ag/AgCl/amp	Hx/fish	–	0.1–10	98	0.1	–	42 days	[58]
Os-gel–HRP/XOD/GCE	0.0 or −0.20 V vs. Ag/AgCl/FI	Hx	–	0.5–80	82.3	0.2	1.1 (n = 20)	6 days	[55]
PANI–XOD–NaMont/MV/CPE	−0.72 V vs. SCE/amp.	Hx/fish	–	1–400	–	0.8	–	14 days (1–5 °C)	[27]
Nafion/XOD/PPh/CFME	0.60 V vs. Ag/AgCl/amp.	Hx/cell culture	–	5–1800	0.031	1.5	1.5 (n = 20)	14 days	[23]
Nafion–GOD/XOD–$PdIrO_2$/GCE	0.70 V vs. Ag/AgCl/amp., HPLC	Glu, Hx/X rat brain	–	1.0–500 (Hx)	–	0.2	4.2 (n = 11)	20 days (4 °C)	[54]
XOD-GA-BSA-nAu-CPE	0.6 V vs. Ag/AgCl/amp.	Hx/ sardines, chicken	0.18	1–100	18.9	0.22	3.9 (n = 10)	15 days	[45]
XOD-HRP-Nano-$CaCO_3$	0.55 V vs. Ag/AgCl/amp	X	–	2–250	171.3	2	4.8	10 days	[34]
s-gel-XOD-CNT-GCE	−0.465 V vs SCE/amp	X	–	0.2–10	–	0.2	–	90 days	[38]
Si-gel-XOD-CPE	0.1 V vs. Ag/AgCl/amp./FI	X	25	2.2	–	2.2	1.2	–	[39]
Bi(III)-XOD-CPE	−0.7 V vs. Ag/AgCl/amp	X/ Beverage		0.02–0.06, 1–7.5		0.02,1	3.84 (n = 25)	7 days 4 °C	[40]
XOD-ZnO-NPs–PPy-PtE	0.4 V vs. Ag/AgCl/amp.	X/fish	13.51	0.8–40	–	0.8	–	100 (40% loss) days	[32]
PVC-BSA-GA-XOD-PtE	0.4 V vs. Ag/AgCl/amp	X/fish	0.45	0.025–0.4	–	0.024	–	45 (30% loss) days	[24]
XOD-ZnO-NP-CHIT-c-MWCNT-PANI-PtE	0.5 V vs. Ag/AgCl/amp	X/fish	–	0.1–100	–	0.1	–	30 days (30% loss)	[42]
XOD-GR	0.6 V vs. Ag/AgCl/amp	X/tea, coffee, fish	0.4	0.1–0.6	–	0.1	–	30 days	[30]

PtE: platinum electrode.
GCE: glassy carbon electrode.
CPE: Carbon paste electrode.
CFME: carbon fiber microelectrode.
NPs: nanoparticles.
PMBQ: poly(mercaptobenzoquinone).
Au coll: colloidal gold.
GA: Glutaraldehyde.
MV: methyl viologen.
FCAPPy: ferrocine carboxylic acid poly(pyrrole).
ITO: indium tinoxide glass electrode.
NaMont: sodium montmorillon.
PPy: Polypyrrole.
PB: Prussian Blue.
PANI: Polyaniline.
CHIT: chitosan, cMWCNT:carboxylated multiwalled carbon nanotubes.
GR: Graphite Rod.
X: Xanthine, Hx; Hypoxanthine.
FiB: Fibre.
BSA: Bovine serum albumin, XOD: Xanthine oxidase.

nylon mesh [20,21], cellulose acetate membrane [22], silk membrane [23], PVC membrane [24] and silk fibroin membrane [25].

Merits: Artificial membranes are high selective for certain bioelements, amplify their responses, because of their higher degree of flexibility, mechanical durability, wider pH range for utilization and higher specific activity.

2.4.2. Polymeric matrices based xanthine biosensors

Polymers have been used widely in the field of electronic sensors, specially in biosensors, due to their chemical and physical properties tailored over a wide range of characteristics [49]. The suitably chosen polymeric matrices are found to be biocompatible, flexible and cost-effective. Besides it, these can be obtained in the form of free-standing films for the fabrication of biosensors [50].

(i) *Xanthine biosensors based on conducting polymer matrices.*

Conducting polymers are the conjugated polymers, which can be synthesized by both chemical and electrochemical methods. These polymers provide easy modulation of various properties (e.g., film thickness, conductivity, functionalization and use of various supporting electrolytes and the ability of serving as an electrochemical transducer itself).

Supports used for immobilization of enzyme: Poly(mercapto-p-benzoquinone) film modified GCE [51], PPy-PtE [52], didodecyldimethylammonium bromide (DDAB) tetrathiafulvalene (TTF) [53], PB-PPy-Au-coll [54], ZnO-NPs–PPy-PtE [32], c-MWCNT-PANI-PtE [31], AuPPy-PtE [29].

Merits: Polymer matrices, as supports, provide enhanced speed, sensitivity and versatility in measurements of desired analytes. Additional merits of polymer film include entrapping of enzyme molecules during electropolymerization in one step and also uniform covering of the surface of substrate electrodes of any shape or size.

2.4.3. Sol–gel-based xanthine biosensors

Sol–gel matrices are known for their rigidness, chemical inertness, thermal and photochemical stability, negligible swelling in aqueous solution, tunable porosity and optical transparency. Besides it, these have been used widely in the fabrication of chemical optoelectronic sensors, as most of the biological materials tend to retain their activity owing to the attractive process of immobilization for various biomolecules (e.g., enzymes and antibodies) at low temperature.

Sol gel supports used for immobilization of enzyme: Os-gel–HRP/XOD/GCE [55], XOD-Gel-GrE [41], s-gel-XOD-CNT-GCE [38], Si-gel-XOD-CPE [39].

2.4.4. Nanomaterials based xanthine biosensors

Nanoparticles (NPs) based xanthine biosensors: Nanoparticles (NPs) based xanthine biosensors have many advantages both in terms of stability and in promoting the catalytic reduction of redox species. NPs have attracted much interest owing to their unique properties including high mechanical strength, oxygen ion conductivity, biocompatibility and retention of biological activities [32]. NPs have electroactive surface of electrode, resulting in enhanced electron transport between electrolyte medium and the electrode.

Nanomaterials used for immobilization of enzyme: The following nano-materials have been used in construction of XOD electrode:XOD/c-M-WCNT/GCE [40], XOD-Au coll-GCE [11], Au-NPs/GC [36], XOD-HRP-Nano-$CaCO_3$ [34], ZnO-NPs–PPy [32], Bi(III)-XOD-CPE [40], XOD-ZnO-NP-CHIT-c-MWCNT-PANI-PtE [42], XOD-AuPPy-PtE [29].

Merits: NPs of metals such as Au, Ag, Pt and Cu, have large surface area, excellent conductivity and catalytic properties, making them suitable as carriers for immobilization of enzymes and active materials to fabricate improved modified electrodes. Among these metal NPs, AuNPs have been used widely to immobilize enzymes, due to their good biocompatibility of for enzymes.

2.5. Nonenzymatic xanthine sensor

A sensitive nonenzymatic amperometric xanthine sensor was constructed based on carbon nanofibers (CNFs). The CNFs, were prepared by electrospinning technique and subsequent thermal treatment, and electrodeposited onto carbon paste electrode (CNF-CPE) to construct an amperometric sensor device without any oxidation pretreatment. The CNF-CPE exhibited high electrocatalytic activity and fast amperometric response. Under the optimal working conditions, the sensor showed a linearity for xanthine in the range 0.03–21.19 μM ($R = 0.9992$) with a detection limit of 20 nM (S/N = 3). With good selectivity and sensitivity, the sensor was successfully applied to study the freshness of fish and determine xanthine in human urine, this provided potential application of sensor to determine food quality control and clinical analysis [48].

2.6. Applications of xanthine biosensors

Xanthine biosensors have been employed successfully for monitoring the meat freshness in fishes [18,29,31,32,36], chicken, pork, beef [29], quantifying the content of xanthine in tea and coffee [30] and measuring the level of serum xanthine required for diagnosis and medical management of xanthineuria, hyperuricemia, gout and renal failure [2].

A comparison of analytical characteristics of various xanthine biosensors [51–58] is summarized in Table 1 and Table 2.

3. Conclusion

In conclusion, xanthine biosensors are strong tool/device for assessing food quality and clinical analysis of several merabolic disorders. Compared to traditional chromatographic and other methods, the strength of the biosensor is that these are very simple selective, sensitive, disposable, work with complete automation and provide rapid results. In the present review, attempts were made to summarize the salient features of various xanthine biosensors reported so far. Most of the reports were focused on the use of XOD for detection of xanthine in biological materials specifically in meat samples. The role of matrices for biosensing and the characteristics of various biosensors, in terms of response time, detection limit and linear range, was delineated.

4. Future perspectives

The applications of nanomaterials such as ZnONPs, AuNPs, Pyrrole and CNT in construction of XOD-based electrochemical biosensors showed the improvement in their analytical performance. To construct a biosensor with promising applications, it should be carefully considered to modify electrode in an effective way. The immobilization of enzyme onto the electrodes should be considered as another key step due to the important roles of the immobilized XOD in the performance of xanthine biosensors. There are many challenges currently faced towards practical applications of xanthine biosensors.e.g the construction of a biosensor with a low cost is still awaited. The major application of xanthine biosensors in medical diagnostics with commercial devices requires to be explored. The biosensors in other areas, such as food industry needed to be explored deeply for more applications. Challenges also exist to find ways to improve the performance criteria including high sensitivity, wider linear range and low limit of detection, fast response and repetitive ability.Further research work is required

to investigate more effective ways to construct XOD based electrochemical biosensors with improved analytic performance.

In future, the development of xanthine biosensors demands for a portable and cheap biosensor with multifunction i.e. detection of other target metabolites/analytes such as hypoxanthine and uric acid beside xanthine for more practical applications. Miniaturization of xanthine biosensor will play an important role in the commercialization of this biosensor in future. However, it may result in low current, because of the low amount of enzyme immobilized onto the available active area of small electrode. This can be overcome by the use of nanomaterials, which enhance the sensitivity of a biosensor by 1–2 orders of magnitude, because of their large surface area per unit volume, which allows the immobilization of a larger amount of the enzyme. Overall, electrochemical biosensors with perfect performance towards commercial systems had a main thrust for future research.

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