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Review

A review of enzymatic uric acid biosensors based on amperometric detection

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ABSTRACT

This review summarizes the studies carried on the development of amperometric uric acid biosensors over the past twenty years. Sensing principles, enzyme immobilization techniques, the electrode types, different approaches and various matrices used for biosensor fabrication are presented along with their benefits and limitations. Uric acid biosensors based on different modes of transducing devices such as optical, potentiometric, conductometric are also referred.

Contents

1. Introduction

Uric acid (2,4,6-trihydroxypurine) is an end product from purine derivatives in human metabolism [1,2]. Uric acid undergoes no further metabolism in humans and is excreted by kidneys and intestinal tract. Serum concentration of uric acid is controlled by the balance of production and excretion [3].

The normal level of uric acid in serum is between 240 and 520 μM and 1.4 and 4.4 mM in urinary excretion [4]. Abnormal uric acid level in biological fluids is a marker of several disorders such as gout [5,6], renal disease [7] and Lesch–Nyhan syndrome [8]. Excessive amounts of uric acid in serum is known as hyperuricemia and this has been found to be associated with hypertension [9,10], metabolic syndrome [11] and cardiovascular disease [3,12–14]. Consequently, fast and reliable determination

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of uric acid in biological fluids is routinely required for diagnosis and treatment.

The first method developed for uric acid analysis was introduced by Offer in 1894. This method is based on the chemical oxidation of uric acid to allantoin, which reduces phosphotungstic acid to a tungsten blue chromophoric compound. However, this method suffers from several problems especially the problem of interferences due to other species capable of producing the same reaction [15]. A more selective approach is the use of uricase enzyme (UOX), which catalyzes the oxidation of uric acid to allantoin, H_2O_2 and CO_2. In 1941, Bulger and Johns [16] introduced a method for the determination of uric acid based on the use of uricase enzyme. This method is based on the determination of the reduction, by protein-free filtrates of an alkaline ferricyanide solution, before and after the destruction of uric acid by uricase. Alternative methods for uric acid determination have appeared since then and various techniques such as chemiluminescence [17–19], fluorescence [15], spectrophotometry [20], HPLC–mass spectrometry [21], ion chromatography [22], high-performance liquid chromatography (HPLC)/isotope dilution mass spectrometry (ID-MS) [23], capillary electrophoresis–amperometry [24], capillary electrophoresis with chemiluminescence detection [25], colorimetry [26] and enzymatic test-kits have been reported. However, these methods are usually laborious, expensive, time-consuming and/or complex to perform. Therefore, there is great interest in developing inexpensive, simple and rapid methods for uric acid determination as a routine analysis. Among these techniques, the enzymatic-colorimetric method using uricase and peroxidase together is widely used in routine analysis due to its simplicity, sensitivity and specificity. Although test kits of this method are commercially available, the cost of uricase and peroxidase used in the kit is a factor that limits widespread use of the method for large number of samples.

Electrochemical methods for uric acid sensing can be classified as nonenzymatic and enzymatic methods. Uric acid is an electrochemically active compound and can be easily oxidized at common electrodes in aqueous solutions to give allantoin and CO_2 [27]. Differential pulse voltammetry [28,29], square wave voltammetry [4,30,31], cyclic voltammetry [32], chronoamperometry [33] are commonly used for the electrochemical detection of uric acid.

Various electrochemical sensors such as zinc–nickel nanoalloy coated composite graphite [32]; conducting polymer modified glassy carbon electrode [34]; nafion coated carbon paste electrode [35]; gold nanotubule electrode [36]; nickel hexacyanoferrate nanoparticles and multiwall nanotube modified glassy carbon electrode [37]; cysteine modified gold electrode [38]; graphene and poly(acridine red) modified glassy carbon electrode [39] have been developed for the determination of uric acid. These methods seem to be more sensitive, simple and less time-consuming than many of the other methods. Nevertheless, uric acid and ascorbic acid commonly coexist in biological fluids of humans, mainly in serum, blood and urine [28]. The major problem with electrochemical determination of uric acid is the closeness of the oxidation potentials of ascorbic acid and uric acid which results in an overlapped voltammetric response and poor selectivity [40]. Several electrochemical approaches including modified electrodes have been purposed to seperate the oxidation peaks of ascorbic acid and uric acid and reduce the interference of ascorbic acid. For instance glassy carbon electrode modified with *L*-cysteine monolayer assembled on gold nanoparticles [41]; conductive poly(3,4-ethylenedioxythiophene) film modified gold electrode [42]; screen-printed carbon electrode modified with nanoplatelets of graphitic oxide [43]; positively charged organoclay film and ferrocene modified glassy carbon electrode [44] were reported for the simultaneous determination of uric acid and ascorbic acid. However, most of the nonenzymatic studies for sensing uric acid showed a lower oxidized potential of ascorbic acid for comparing with uric acid. The results showed that the interference caused by ascorbic acid for monitoring uric acid with the anodic oxidation technique is still a problem. Other electrochemically oxidizable species such as dopamine and xanthine can also interfere with the analysis of uric acid. Therefore, various studies for the simultaneous determination of these species were reported [45–48].

Selectivity and sensitivity of electrochemical uric acid determination can be improved by enzyme-based electrochemical methods. The use of biosensors, which allow direct, specific, sensitive, rapid and inexpensive measurement of uric acid in samples, seems to be one method of choice. Since the initiator work of Clark and Lyons [49] there has been a rapid growth in research activities in this area and biosensors for various other substances have been developed and utilized in many applications in food industry, clinical chemistry or environmental control, industrial process control and biological warfare agents [50–52]. Biosensors can be classified as electrochemical, optical, calorimetric and piezoelectric based on the transduser used [53–55]. In electrochemical biosensors, the signal produced by the interaction of a biological element and substrate can be measured by an electrochemical detector [56]. Calorimetric biosensors are based on the measurement of heat accompanying a biochemical reaction [57]. Piezoelectric biosensors are mass sensitive and detect the change in frequency of oscillation after adsorption or desorption of analyte molecules on the surface of piezoelectric detector [58]. Optical biosensors are based on the measurement of adsorbed or emitted light resulting from a biochemical reaction [59]. Biomolecules such as enzymes, cofactors, antibodies, nucleic acids, tissues, receptors, organelles, microorganisms and animal and plant cells have been used as biological recognition elements [55,60–63]. Among these biological elements, enzymes are the most widely used recognition element due to their high selectivity, specificity and sensitivity [64].

This review aims to provide an overview of uric acid biosensors based on amperometric detection. The advantages and disadvantages associated with the electroactive species detected are discussed in detail. The enzymes widely used in uric acid biosensors, sensing principles, electrode types, various matrices for biosensor construction and enzyme immobilization techniques are also referred.

2. Amperometic detection

Amperometric enzyme electrodes for uric acid determination are generally based on the determination of enzymatically generated H_2O_2 or the consumption of O_2 during the enzymatic reaction [65–69]. Uric acid determination based on H_2O_2 detection has received considerable interest because H_2O_2 can be detected either by its reduction or oxidation.

Uricase and horseradish peroxidase (HRP) enzymes are widely used in amperometric uric acid biosensors. Uricase (urate oxidase; EC 1.7.3.3) catalyzes the oxidation of uric acid to allantoin, CO_2 and H_2O_2 according to the reaction:

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{UOX}} \text{Allantoin} + H_2O_2 + CO_2 \quad (1)$$

The enzyme exists as a tetramer composed of two types of different subunits [70]. The enzyme has been found in mammals [71,72], plants [73,74], fungi [75], yeast [76] and bacteria [77].

The degradation of uric acid varies from species to species. In humans and many other primates uricase is missing. It has been reported that the enzyme has been lost by some unknown mechanism during primate evolution [71]. Thus, uric acid is the

end product of purine catabolism in humans. Most other mammals contain urate oxidase and allantoin is excreted as the end product of purine metabolism. Birds and reptiles are also devoid of uricase, and excrete uric acid as the final product of purine degradation. Microorganisms such as bacteria and yeasts further catalyze the oxidation of uric acid to allantoin. Allantoin is hydrolyzed by allantoinase (EC 3.5.2.5) to form allantoic acid and allontoic acid is converted to urea by allantoicase (EC 3.5.3.4). The enzyme urease (EC 3.5.1.5) then converts urea to ammonia and carbon dioxide [78]. In fish liver, urate oxidase, allantoinase and allantoicase are present and uric acid is degraded to urea [79].

It has been proposed that, the loss of uricase in humans has been advantageous since uric acid is an antioxidant and scavenger of free radicals. The presence of a high serum uric acid level due to the loss of uricase activity protects the body from oxidative damage and this may decrease the cancer rate and prolong the lifetime. Another view suggests that the loss of uricase is responsible for many metabolic disorders [71]. Because an abnormal increase of human uric acid level is a causative factor of gout and many other diseases including renal problems and Lesch–Nyhan syndrome [6–8].

In uric acid biosensors uricase from different sources such as *Arthrobacter globiformis* [80], *Bacillus fastidiosus* [81], *Aspergillus niger* [82] and *Candida sp.* [83] is used as the biosensing element. The dependence of the biosensor response on the type of the uricase enzyme was also reported [84]. The uric acid biosensor based on uricase from *Candida Utilis* showed the best response while the one based on uricase from porcine liver did not show a detectable signal due to the low specific activity of the enzyme.

Horseradish peroxidase (HRP) (EC 1.11.1.7), a heme containing glycoprotein extracted from horseradish root, is the most commonly used peroxidase in analytical applications [85]. In biosensor studies many substrates have been determined using oxidase and peroxidase coupled reactions [86–88]. The oxidase enzyme oxidizes the substrate to an oxidized product and hydrogen peroxide, in the presence of oxygen. The produced hydrogen peroxide can be measured amperometrically. Hydrogen peroxide can be electrooxidized at platinum electrode with applied potential of +0.70 V (vs. SCE). At this high potential, other electroactive species present in the sample can also oxidize and interfere with the analysis. The use of peroxidase leads to the enzymatic reduction of H_2O_2 at lower potentials which decreases interference effects [66]. The use of HRP in amperometric uric acid biosensors is discussed in Section 2.2.

2.1. O_2 detection

O_2 monitoring is typically based on a Clark type O_2 electrode consisting of a platinum cathode, where O_2 is reduced, and a reference electrode, usually Ag/AgCl. When a potential of -0.60 V versus Ag/AgCl is applied to the Pt electrode, O_2 is reduced according to reaction (3), and a current proportional to O_2 concentration is produced.

$$\text{Ag anode, } Ag + 4Cl^- \rightarrow 4AgCl + 4e^- \quad (2)$$

$$\text{Pt cathode, } O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (3)$$

The first amperometric method for quantitative uric acid determination in biological fluids was reported by Nanjo and Guilbault [89] in 1974. In this method a platinum electrode covered with an immobilized uricase is used for the direct measurement of dissolved oxygen consumption at −0.60 V.

A uric acid biosensor using dithiothreitol (DDT), a reducing agent, to amplify the consumption of oxygen caused by the uricase reaction was developed [90]. In this study DDT reacts with the ternary complex (uricase, uric acid and oxygen) and uric acid is regenerated via the ternary complex accompanied by an amplified consumption of oxygen. A biosensor based on uricase and peroxidase enzymes for uric acid determination in urine was reported [65]. The method is based on the consumption of H_2O_2 by peroxidase and measurement of the decreasing of dissolved oxygen concentration. The results for uric acid concentration in urine obtained by using the biosensor were reported to be more sensitive and accurate than the results of the enzymatic kit; however the linear working range of the biosensor was narrow. No response of the common interfering compounds was reported. Uchiyama and Sakamoto [91] immobilized uricase on a porous carbon felt by electropolymerization of pyrrole or aniline. The enzyme immobilized carbon felt was fixed on the gas permeable membrane of the Clark electrode and consumed oxygen was detected. The carbon felt reported to be an excellent matrix for uric acid sensing due to its porosity permitting a transfer of oxygen. A uric acid biosensor prepared from uricase immobilized eggshell membrane and oxygen electrode was reported [92]. The enzyme immobilized eggshell membrane was placed on the surface of the oxygen sensor and was kept in a steady position by an *O*-ring. In this method the decrease in oxygen level was monitored. Interferents such as glucose, urea, ascorbic acid, lactic acid, glycine, KCl, NaCl, $CaCl_2$, $MgSO_4$, and NH_4Cl showed no interferences on the response of the uric acid biosensor. Uricase was immobilized onto an epoxy resin membrane and the membrane was positioned over the sensing part of the combined electrode of dissolved O_2 meter [93]. The epoxy membrane was reported to be an ideal material for an enzyme electrode due to its high affinity for enzyme, high temperature stabiliy, porosity, low cost and chemical resistance. Glucose, urea, NaCl, cholesterol and ascorbic acid caused a decrease in activity, while, no effect of $MgSO_4$ and $CaCl_2$ was observed. The surface of a gold (Au) electrode was coated with uricase immobilized polystyrene membrane [94]. The uric acid determination was based on the decrease in the reduction current of oxygen and the interference of ascorbic acid was eliminated. Uricase immobilized silk fibroin membrane and an oxygen electrode in flow injection analysis was reported for amperometric uric acid analysis [95]. Interference effect of ascorbate, glucose, uric acid, urea, glutamic acid, sucrose and lactose was investigated and only interference of uric acid, lactose and ascorbate was observed.

In oxygen determination based studies, interference effect is not very common; however, the response of the electrode depends on the oxygen concentration in the solution and this can reduce the accuracy of the biosensor. The most commonly used alternative to overcome this drawback is the detection of H_2O_2. Table 1 presents a detailed comparison of significant characteristics of uric acid biosensors based on O_2 detection.

2.2. H_2O_2 detection

Enzymatically produced H_2O_2 can be detected electrochemically by amperometric electrodes, either by measuring the anodic or cathodic response, due to the oxidation (4) or reduction (5) of H_2O_2 at the surface of the working electrode respectively.

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (4)$$

$$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O \quad (5)$$

Electrochemical oxidation of H_2O_2 requires high potentials (> 0.40 V). Other electroactive species (e.g. ascorbic acid, uric acid) present in the sample can also oxidize and interfere with the analysis at high potentials [66]. In the case of amperometric uric acid biosensors the most important drawback is the possible oxidation of uric acid itself-as well as of the other electroactive species present in the sample at the electrode surface such as Pt,

Table 1
Characteristics of uric acid biosensors based on oxygen detection.

Enzyme, immobilization technique and matrix used	Uricase source	Detection limit	Linear range	Sensitivity	Stability	Response time	Applications	Ref.
UOX-HRP, glutaraldehyde crosslinking, teflon membrane	Porcine liver	0.1 μM	0.1–0.5 μM	–	17% loss after 1 month	–	Uric acid in urine	[65]
UOX, electropolymerization, porous carbon felt	*Candida Utilis*	4×10^{-6} M	1×10^{-5}–4×10^{-4} M	4.74 nA μM–1 cm^{-2}	5–7 days	3 min	Uric acid in serum	[91]
UOX, glutaraldehyte cross-linking, egg shell membrane	*Arthrobacter globiformis*	2 μM	4–6.4 μM	–	3 months	<100 s	Uric acid in serum and urine	[92]
UOX, hydrogen bonding, epoxy resin membrane	Blacked eyed cowpea leaf	4.25 μg/mL	0.025–0.10 mM	–	32% loss after 60 days	10–12 s	Uric acid in serum	[93]
UOX, spin coating, polystyrene membrane	Yeast	–	5–105 μM	–	13% loss after 21 days	–	–	[94]
UOX, entrapment, silk fibroin membrane	Porcine liver	–	0.2–1.0 mmol/L	–		50 s	Uric acid in serum and urine	[95]

Table 2
Characteristics of uric acid biosensors based on H_2O_2 detection.

Enzyme, immobilization technique and matrix used	Uricase source	Detection limit	Linear range	Sensitivity	Stability	Response time	Applications	Ref.
UOX-HRP, entrapment, carbon paste covered with poly(o-aminophenol)	–	3×10^{-6} M	Up to 1×10^{-4} M	–	2 days	37 s	Uric acid in serum	[66]
UOX, glutaraldehyte cross-linking, polypyrrole membrane	Microorganism	5×10^{-7} M	$1\times10^{-6}-5\times10^{-5}$ M	–	51% loss after 7 week	330 s	Uric acid in serum and urine	[96]
UOX, entrapment, carbon paste	*Arthrobacter globiformis*	1.9×10^{-7} M	$1\times10^{-6}-1\times10^{-4}$ M	–	13% loss after 4 months	70 s	Uric acid in serum	[97]
UOX, cross-linking, self-assembled monolayer of 2-aminoethanethiolate on gold electrode	–	–	Up to 6×10^{-6} M	143 nAμM^{-1} cm^{-2}	1 week	2 min	–	[98]
UOX, physical adsorption, NiO film	–	0.11 mM	0.05–1.0 mM	1278.48 μA/mM	About 4 months	5 s	–	[99]
UOX-HRP, cross-linking, cylindrical pencil lead	*Arthrobacter globiformis*	5.9×10^{-7} M	$5.9\times10^{-7}-1.2\times10^{-4}$ M	–	10 days	–	Uric acid in serum	[101]
UOX, Langmuir–Blodgett technique, chitosan/Prussian blue film	–	1.8×10^{-7} M	$5\times10^{-6}-1.15\times10^{-3}$ M	–	20%loss after 2 weeks	5–10 s	Uric acid in urine	[103]
UOX, layer-by-layer, polyelectrolyte multilayer film composed of poly(allylamine) and poly(vinylsulfate).	*Candida sp.*	–	$1\times10^{-6}-1\times10^{-3}$ M	–	40% loss after 30 days	–	–	[106]

Au and carbon at high potentials, at which the electrochemical oxidation of H_2O_2 occurs [66,96]. Furthermore, H_2O_2 detection by its reduction can suffer from the interference of oxygen. To overcome the drawbacks encountered with the direct H_2O_2 detection, alternative routes to follow the production of H_2O_2 have been proposed.

A way to decrease the applied potential and thus the interference effect is the use of mediators instead of oxygen. A carbon paste enzyme electrode for uric acid determination was prepared with uricase and tetracyanoquinodimethane as mediator [97]. Working potential of the electrode was +0.34 V and it was reported that this low potential minimized the interference effect of other electroactive species as well as overcame the direct oxidation of uric acid itself. Ferrocene was dissolved in electrolyte solution and was investigated as an electron transfer mediator [98]. Enzyme immobilized Au electrode exhibited amperometric response to uric acid at a low working potential of +0.10 V by the use of this mediator. A uric acid biosensor was developed by the electropolymerization of pyrrole and electron mediator ferrocene on Pt electrode surface was reported [96]. Uricase was immobilized onto the polymer film due to crosslinking by glutaraldehyde. In this study interference effects of ascorbic acid, uric acid, paracetemol and methionine was mentioned at +0.70 V. Interfering effects were eliminated by the dilution of the sample. Uricase was immobilized onto the NiO thin film and this film was deposited on a platinum coated glass substrate for the development of uric acid biosensor [99]. $[Fe(CN)_6]^{3-/4-}$ was used as a mediator for electron transfer from the enzyme to the electrode via NiO thin film matrix. Interference effect of glucose, cholesterol, urea, ascorbic acid and dopamine was investigated and maximum interference of 2.5% was reported. Copper oxide (CuO) thin films were grown onto platinum (Pt) coated corning glass substrates by pulsed laser deposition technique for the construction of a uric acid biosensor. In this work the CuO thin film matrix was reported as an efficient electron conducting platform due to the redox properties of Cu^{+}/Cu^{2+} couple. No significant effect of common interferants, like cholesterol, glucose, lactic acid, urea, and ascorbic acid, was reported on the performance of the uricase/CuO/Pt/glass electrode toward uric acid sensing [100].

Another approach to lower the working potential is the use of HRP together with uricase and to perform uric acid determination via H_2O_2 reduction. A reagentless uric acid biosensor was purposed by the immobilization of uricase and horseradish peroxidase in the carbon paste matrix [66]. Poly(aminophenol) was electropolymerized at the working surface of the electrode acting as the conducting polymer layer. This electrode did not suffer from the common interfering substances at an applied potential of +0.05 V. A mediated amperometric biosensor for the determination of uric acid in human serum was described [101]. UOX and HRP were co-immobilized by crosslinking with glutaraldehyde.

Ferrocenecarboxylic acid was used as the mediator and the working potential was +0.05 V. In the interference study no effect was observed due to the electroactive species and this was attributed to the low working potential.

It was reported that Prussian blue acts as an artificial peroxidase for electroreduction of H_2O_2 [102]. A uric acid biosensor based on chitosan (CS) and Prussian blue (PB) prefunctionalized indium–tin oxide (ITO) electrode was described [103]. Uricase was immobilized onto the modified electrode by Langmuir–Blodgett (LB) technique [104]. CS–PB was reported as a good electrocatalyst for the reduction of hydrogen peroxide produced by enzymatic reaction of UOX. Iveković et al. [105] developed a uric acid based on alkaline-stable H_2O_2 transducer. Prussian blue was electrochemically deposited on graphite disc electrode and Ni^{2+} ions were incorparated into modified electrode. Uricase was immobilized on the surface of the modified electrode by crosslinking. In this work Ni^{2+}-modified Prussian blue was used as a catalyst for the reduction of enzymatically produced H_2O_2.

The use of H_2O_2 selective membranes is another approach to decrease the effect of interferences. Such a method was described by Hoshi et al. [106] using polyelectrolyte multilayer film composed of poly(allylamine) and poly(vinylsulfate). The purposed membrane functions as a permselective film in which H_2O_2 can pass through while interfering substances such as uric acid and ascorbic acid cannot. Keedy and Vadgama [107] immobilized uricase by crosslinking a dimethyldichlorosilane treated microporous polycarbonate membrane and an inner cellulosic H_2O_2 selective membrane for uric acid enzyme electrode preparation. Table 2 presents a detailed comparison of significant characteristics of various uric acid biosensors based on H_2O_2 detection.

3. Enzyme modified electrodes

3.1. Immobilization techniques

Enzyme immobilization is the key step in the construction of biosensors. The choice of the immobilization method depends mainly on the enzyme to be immobilized, the immobilization matrix and the transducing mechanism. Immobilized enzymes have many advantages such as good thermal and operational stability, reusability, continuous use, easy separation of the enzyme from product and reduced cost of operation [108]. The main disadvantage of the immobilized enzymes is the possible loss of enzyme activity during immobilization. Thus, many studies on immobilization techniques to enhance the operational and storage stability of the enzymes were reported. Immobilization can be carried out using many different procedures, such as physical adsorption, entrapment, cross-linking and covalent linking. Each immobilization method has advantages and disadvantages.

Adsorption and entrapment are the widely used physical methods for enzyme immobilization. Physical adsorption is the oldest and simplest method for enzyme immobilization. In this method the enzymes are immobilized due to weak binding forces such as Van der Waal's forces, hydrogen bonds and electrostatic interactions. However, these binding forces are susceptible to changes in pH, temperature and ionic strength. The operational and storage stability of the biosensors based on adsorbed enzymes is not satisfactory due to enzyme desorption. Enzymes can be immobilized by physical entrapment into gel, polymer or carbon paste matrices. Entrapment usually enhances the stability of the biosensor. Possible leakage of enzymes from the matrix and transport limitations offered by the entrapment material are the main drawbacks of this method. Covalent binding and cross-linking represent the chemical methods of enzyme immobilization. Covalent binding involves the formation of a stable covalent bond between functional groups of the enzymes and the support matrix. The surface of the support is generally activated with multifunctional reagents such as glutaraldehyde, tosyl chloride, carbonyldiimidazole and carbodiimide followed by the coupling of the enzyme to the activated surface. In covalent binding the strength of binding is very strong; thus, leakage of enzyme is limited. However, low reproducibility is the major limitation of the biosensors based on covalent binding. Crosslinking using bifunctional reagents like glutaraldehyde or carbodiimide is another effective and widely used method for enzyme immobilization. The major limitation of this method is the possibility of activity losses due to the distortion of the active enzyme conformation and chemical changes in the active site of the enzyme by the bifunctional reagents [109–113].

3.2. Polymer based electrodes

Polymer matrices can be used both in the sensing mechanism and in the immobilization of the enzymes. Polymeric films are widely used for the immobilization of enzymes. Different types of conductive, nonconductive or composite polymeric films were purposed for amperometric biosensor designs [109,114]. Here, some selected examples of polymer based amperometric uric acid biosensors will be presented.

Conducting polymers, like polyaniline (PANi) and polypyrrole have been widely used for the fabrication of amperometric uric acid biosensor. An amperometric uric acid biosensor based on uricase immobilized in polypyrole film was reported [96]. Among other conducting polymers, polyaniline is often used as the immobilizing substrate for enzymes and as an efficient electrocatalyst. A simple and cheap procedure to immobilize enzyme into the polyaniline film was described by Mu [115]. In this one step process enzymes were entrapped into the polmer film directly. It was reported that such a process can cause enzyme activity to be affected by the aniline monomer in the solution. Another way to immobilize enzymes into polyaniline film is doping of the enzymes after the polymerization process in a two step process [116]. However it was reported that the stability of the immobilized enzymes can be affected by the distance between the particles and if the particle distance of the polymer is shorter than the diameter of the enzyme molecule enzymes are only adsorbed on the surface of the film and can easily desorb. In another study Kan et al. [67] immobilized uricase into polyaniline film by template process. In this process a polyaniline–uricase electrode was obtained by electropolymerization. The electrode was then hydrolized to remove the uricase that might be affected by aniline monomer from polyaniline film. Active uricase was immobilized into the polymer film by doping. This method overcomes the effect of the monomer on enzyme activity and avoids the stability problems due to the cavities of the polymer. Arora et al. [117] prepared an amperometric uric acid biosensor by the deposition of polyaniline on ITO glass substrates. Uricase was immobilized onto the glutaraldehyde activated polymer surface by crosslinking. Glutaraldehyde covalently binds with the end amine groups of the polyaniline film and the other end of the glutaraldehyde binds with the amino group of the enzymes. No significant effect of interferences on the response of the purposed biosensor was reported. A uricase biosensor based on poly-o-aminophenol-aniline was reported [118]. Uricase was immobilized into the copolymer by the template process [67]. The stability of the purposed biosensor was found to be higher than the one prepared by the two-step process [119]. Arslan [120] prepared a polyaniline–polypyrole composite film for the estimation of uric acid. Uricase was immobilized on the composite film by crosslinking with glutaraldehyde. Interfering effect of paracetemol, glucose, urea, bilirubin and ascorbic acid was investigated and only the interference of paracetemol (31%) was reported.

The layer-by-layer (LBL) method is a simple and cheap thin film fabrication technique. Film fabrication by the LBL method is performed under mild conditions, which is important for minimizing protein denaturating and preserving biomolecule activity. Moraes et al. [121] described a uric acid biosensor fabricated by LBL technique. Indium tin oxide (ITO) glass substrate was modified with a Prussian Blue (PB) layer. Uricase enzyme was immobilized onto modified ITO substrates by layer-by-layer technique. Poly(ethyleneimine) or poly(diallyldimethylammoniumchloride) was used for film construction. The detection of H_2O_2 was carried out at 0.0 V by the use of PB layer. This avoided the direct oxidation of uric acid and eliminated the effect of other interferences.

Polymer membranes can also act as a protective layer, preventing electrochemically interfering compounds such as ascorbic acid, uric acid from reaching the surface of the electrode. Poly (allylamine) and poly(vinyl sulfate) polyelectrolyte multilayer film have been used as an effective barrier against the interference of ascorbic acid and uric acid [106]. After the deposition of the permselective film on the surface of platinum electrode uricase and poly(allyamine) were deposited via layer-by-layer sequential deposition up to 10 Uricase layers to prepare the amperometric biosensor. In this study uric acid determination was achieved in the presence of uric acid and ascorbic acid. Jiang et al. [82] carried out the polymerization of aniline in an ionic liquid, and then uricase was immobilized into the film by a two step process. Polyaniline film was reduced and the reduced film was transferred into the buffer containing uricase then the polymer film was oxidized at a positive potential. The uricase enzyme with negative charge was doped into the polymer film. It was reported that H_2O_2 can permeate through the polyaniline film synthesized in ionic liquid; however the film has no significant catalysis to uric acid. The film was impermeable to interfering substances. An amperometric uric acid has been developed in which polymaleimidostyrene was used as a dispersant for uricase in order to immobilize the enzyme in the polystyrene membrane [94]. A gold electrode was covered with polystyrene membrane by the spin coating method. The membrane was prepared from polystyrene solution and polymaleimidostyrene solution containing uricase enzyme. Uric acid was determined at a negative potential based on the decrease in the reduction current of dissolved oxygen. By the use of the polystyrene membrane inteference effect of ascorbic acid was also eliminated. The work suggests that a homogenous enzyme membrane can only be formed in the presence of polymaleimidostyrene. Uricase is covalently attached to polymaleimidostyrene; polymaleimidostyrene bonds the enzyme and polystyrene membrane and acts as a dispersant for the uricase. 2-Aminophenol was electropolymerized on Pt electrodes and the resulting polymeric film was reported to allow penetration of uric acid while blocking the electrochemical activity of ascorbic acid [122]. No significant effect of interfering substances such as ascorbic acid, cysteine, oxalic acid, lactose, sucrose, and urea was reported. Zhao et al. [123] reported a uric acid based on a hemoglobin encapsulated chitosan modified glassy carbon electrode. Uricase was immobilized on hemoglobin and chitosan modified electrode. Enzymatically produced H_2O_2 was reduced by hemoglobin. No significant interference effect was reported. Uric acid biosensor was applied for uric acid determination in human serum samples.

In polymer based uric acid biosensors drawbacks such as the effect of the monomer on the immobilized enzyme activity, stability of the enzyme, desorption of the immobilized enzyme from the polymer film and mechanical stability of the polymer challenge researchers. Attempts and approaches should be directed toward solving these problems.

3.3. Carbon paste based electrodes

Carbon paste electrodes have been widely used for electroanalytical applications since their introduction by Adams in 1958 due to their advantages such as low background current, low cost of fabrication, good electron transfer kinetics, biocompatibility and ease of modification [124]. A number of uric acid biosensors based on carbon paste matrix containing uricase and in some cases uricase and horseradish peroxidase have been reported [66,97,125]. Erden et al. [125] immobilized uricase into the carbon paste by crosslinking with bovine serum albumin and glutaraldehyde and redox mediator 1,4 benzoquinone was also incorparated into carbon paste. The described electrode showed excellent response to uric acid with very low detection limit. Another redox polymer poly(vinylferrocenium) was also used for amperometric uric acid biosensor by the same group. Poly(vinylferrocenium) was mixed with carbon paste and uricase was immobilized by crosslinking. Among the various interfering compounds investigated, ascorbic acid, glucose, urea and methionine caused significant interference and this effect was eliminated by the dilution of the sample. The use of an additional membrane in the configuration of carbon paste biosensors can also be a good choice in order to prevent undesirable interferences from electroactive species in real samples. Miland et al. [66] co-immobilized uricase and horseradish peroxidase within a carbon paste. Poly (o-aminophenol) was electropolymerized at the surface of the carbon paste acting as the conducting layer. They reported that this polymer coating also protects the electrode from interferences and fouling. Dutra et al. [97] prepared a carbon paste electrode by mixing the graphite powder, electron transfer mediator tetracyanoquinodimethane and uricase with mineral oil. This electrode was incorparated into a flow injection system and 50 measurements/h of uric acid analysis was achieved.

Carbon paste electrodes are prepared by mixing graphite powder and liquid nonelectrolytic binder. The experience of the researcher and the composition of the carbon paste are the main factors that limit the applicability of the carbon paste based biosensor. For example, the amount of the binder has to be optimized carefully since it has a great influence on the sensitivity and reproducibility of the biosensor. In carbon paste based biosensors usually a high amount of enzyme is mixed with the paste which would increase the cost of the biosensor. Moreover, carbon paste based biosensors suffer from mechanical damage during use.

3.4. Nanomaterial based electrodes

Nanomaterials have found a great variety of applications owing to characteristic properties such as large surface-to-volume ratio, high surface reaction activity, high catalytic efficiency and strong adsorption ability that are useful in biosensing applications. Nanomaterials also have the unique ability of promoting fast electron transfer between electrode and the active site of the enzyme [126]. Due to their unique electrochemical, mechanical and structural properties, carbon nanotubes (CNTs) have also aroused much interest as components in biosensors [127,128]. Metal oxide nanoparticles and carbon nanotubes are widely used for the development of uric acid biosensors.

A reagentless amperometric uric acid biosensor based on functionalized multiwall carbon nanotubes (MWCNTs) with tin oxide (SnO_2) nanoparticles was described [129]. In this study, nitric acid treated MWCNTs was immersed in the tin(II) chloride solution which was sonicated and stirred at room temperature. This sample was centrifuged, rinsed with water, filtered and MWCNTs–SnO_2 was dispersed in distilled water. Carboxylic acid functionalized MWCNTs were fabricated by heating at reflux with

HNO_3 and dispersed in distilled water. This dispersed solution was dropped on the surface of glassy carbon electrode and water was evaporated. Uricase was immobilized on the MWCNTs–SnO_2/GCE or MWCNTs–COOH/GCE by drop coating for biosensor construction. It was reported that MWCNTs–SnO_2 based biosensor showed higher electrocatalytic oxidation to uric acid than the carboxylic acid functionalized MWCNTs based biosensor. Common interfering compounds such as ascorbic acid and glucose showed no effect on the biosensor response at low uric acid concentrations. Biosensor was used for uric acid determination in dialysate samples in rat striatum. Chauan and Pundir [130] fabricated an amperometric uric acid biosensor by immobilizing uricase onto gold nanoparticle (AuNP)/multiwalled carbon nanotube (MWCNT) layer deposited on Au electrode. The enzyme was immobilized by carbodiimide linkage. Uric acid determination was based on oxidation of enzymatically generated H2O2 at +0.40 V. AuNPs were reported to enhance the current response and showed good catalytic activity towards H_2O_2 electrooxidation. The current response of MWCNT or AuNP−MWCNT modified electrodes was found to be higher than bare Au electrode due to the increase in effective electrode surface area. The biosensor was used to measure uric acid levels in serum of healthy persons and gout patients. No interference of electroactive compounds such as glucose, cholesterol, urea, pyruvate, bilirubin, $CuSO_4$, KCl, FAD, NaCl, $ZnSO_4$, NADH, $CaCl_2$, EDTA, riboflavin, and $MnCl_2$ was reported. On the other hand ascorbic acid showed interference at its physiological concentration. Rawal et al. [131] fabricated a uric acid biosensor based on uricase immobilized on Prussian blue nanoparticles (PBNPs), carboxylated multiwalled carbon nanotube (cMWCNT) polyaniline (PANI) gold (Au) composite. Aniline was electropolymerized on Au electrode by cyclic voltammetry. The polyaniline (PANI) modified electrode was immersed into the cMWCNT solution to obtain the c-MWCNT/PANI/Au electrode. PBNPs were electrodeposited onto the c-MWCNT/PANI/Au electrode. Uricase was immobilized by crosslinking by chitosan and glutaraldehyde. A good correlation (r=0.99) was reported between serum uric acid values by the standard enzymatic colorimetric method and the presented method. An amperometric uric acid biosensor based on multiwalled carbon nanotubes (MWNTs) and ZnO nanoparticles was described [132]. Pyrolytic graphite (PG) wafers were modified with MWNTs and then ZnO nanoparticles were decorated onto the MWNT films. Uricase was cast on the ZnO-MWNT-PG electrode and dried at room temperature. Enzyme immobilization process was based on the isoelectric point differences of uricase and ZnO nanoparticles. The resulting enzyme electrode was coated with 0.5% polydiallyldimethylammonium chloride (PDDA) to eliminate the possible fouling and to prevent the leaching of the enzyme. Interference effect of ascorbic acid (AA), glucose, and *L*-cystine was investigated by DPV and it was reported that DPV responses of these compounds were successfully separated by the sensor. Bhambi et al. [133] described a uric acid biosensor by the immobilization of uricase onto a polyaniline-multiwalled carbon nanotube composite film by carbonamide linkage. This biosensor was applied to the determination of serum samples of healthy individuals and the persons suffering from hyperuricemia and gout. Zhang et al. [134] reported a uric acid biosensor based on uricase immobilized on ZnO nanorods. The surface of a glassy carbon electrode was modified with Nafion and ZnO nanorods were decorated onto the modified electrode. Uricase was immobilized onto ZnO modified film electrostatically. ZnO nanorods provided a high surface area and enhanced electron transfer between the electrode surface and enzyme. No interference of ascorbic acid (AA), glucose and L-cystine was reported.

Current developments in nanomaterial technology show that nanomaterial based biosensors have a great potential for uric acid determination. These biosensors are generally characterized by higher sensitivities, lower detection limits, and faster electron transfer kinetics in comparison to the other modified electrodes. Therefore, nanomaterials are likely to be a good choice to obtain improved sensing properties in uric acid biosensors.

3.5. Self-assembled monolayer based electrodes

Self-assembled monolayers (SAMs) can be defined as ordered molecular assemblies formed spontaneously by adsorption of an active surfactant on a solid surface. Self-assembled monolayers are usually formed by the chemisorption of a head group that shows a specific affinity towards the substrate, followed by the organization of the tail group which consists of a functional group at the terminal end. Head and tail groups are usually connected through a chain or backbone which is the main structure of the molecule. SAMs of alkanethiolates on gold and silane based systems are the most widely known SAMs [135–137]. In biosensor applications, modified electrodes based on self-assembled monolayers have been extensively used due to their simple, reliable and reproducible formation and preparation. Furthermore SAMs provide a convenient platform to immobilize biomolecules [138].

Kuwabata et al. [98] immobilized uricase by crosslinking with glutaraldehyde to a self-assembled monolayer of 2-aminoethanethiolate prepared on Au electrode substrate. $[Fe(CN)_6]^{3-}$ dissolved in solution was used as electron transfer mediator. Another amperometric uric acid biosensor was based on the microperoxidase-11 (MPx-11) covalently immobilized with 1-ethyl-3-(3-dimethyaminopropyl)carbodiimide on the mixed self-assembled monolayer of 2-(2-mercaptoethylpyrazine) (PET) and 4,4′-dithiodibutyric acid (DTB) on gold electrode [139]. Mixed self-assemblies of PET and DTB were made by immersing the cleaned Au electrode in ethanol solutions containing PET and DTB. MPx-11 acted as a mediator for the reduction of H_2O_2. Uricase was immobilized on the MPx-11 anchored electrode with chitosan. No interference of ascorbic acid and paracetemol was reported both in uric acid and H_2O_2 determination. Ahuja et al. [140] immobilized uricase covalently on a self-assembled monolayer (SAM) of 3-aminopropyltriethoxysilane (APTES) using a crosslinker, Bis[sulfosuccinimidyl] suberate (BS3) on an indium–tin-oxide (ITO) coated glass plate. Amperometric response was measured as a function of uric acid concentration at +0.26 V in phosphate buffer containing $[Fe(CN)_6]^{3-}$. The effective immobilization of the enzyme on the surface of the modified electrode improved sensitivity of the biosensor. Redox compounds such as 5-methylphenazinium (MP) and 1-methoxy-5-methylphenazinium (MMP) were investigated as mediators for UOX [141]. It was reported that MP and MMP were useful electron acceptors for UOX. Poly(N-methyl-o-phenylenediamine) (poly-MPD), a novel redox polymer, containing MP acted as a mediator for UOX. This polymer film was co-immobilized with UOX onto an Au electrode substrate covered with a self-assembled monolayer of 2-aminoethanethiolate. In another study Au substrate was coated with a self-assembled monolayer of n-octanethiolate (OT/Au) [142]. UOx and redox mediator 1-methoxy-5-methylphenazinium (MMP) were dissolved in the suspension of L-α-phosphatidylcholine β-oleoyl-γ-palmitoyl (PCOP) liposomes and fixed in the bilayer of PCOP and *n*-octanethiol. The bilayer blocked the diffusion of ascorbic acid and uric acid and eliminated the interference effect.

3.6. Screen printed electrodes

Screen printed technology can be used for the development of single use, disposable sensors for clinical, environmental and industrial analyses. Screen-printed electrodes (SPEs) are fabricated by printing different inks on various types of solid

substrates. This technique has advantages of simple construction, high reproducibility, low cost, and a wide choice of materials [143]. Among the various screen printed electrodes, screen printed carbon electrodes have gained more interest due to ease of fabrication and modification. However, very little studies have been reported in the area of screen printed carbon electrode based uric acid biosensors. Gilmartin and Hart [144] developed a uric acid biosensor based on cobalt phthalocyanine modified screen printed carbon electrode. The modified electrode was covered with cellulose acetate layer and uricase was immobilized on the cellulose acetate layer by drop coating method. Cellulose acetate film acted as a permselective membrane towards H_2O_2 and electrochemical oxidation of the Co^+ species was evaluated as the analytical signal. This indirect measurement process of uric acid resulted in an application of reduced operating potential when compared with that required for the direct oxidation of H_2O_2 at unmodified electrodes. Luo et al. [81] reported an iridium-modified carbon electrode prepared by the thick film screen printing technique. The bases of working, counter and reference electrodes were prepared by screen printing gold ink for working and counter and silver ink for reference electrode on alumina substrate. The working electrode base was screen printed with Ir–C ink composed of Ir–C powder, polyethylenimine (PIE) and hydroxyethyl cellulose in phosphate buffer solution. Uricase was immobilized onto the surface of the working electrode with the casting method. Glutaraldehyde solution was cast on the electrode surface and glutaraldehyde was covalently bound with the amine group in the PEI; then uricase was immobilized on the electrode by crosslinking. H_2O_2 detection was achieved at $+0.25$ V and a significant interference effect of ascorbic acid and uric acid was not observed at this potential.

A screen-printed carbon electrode modified with electrocatalyst cobalt phthalocyanine was reported as the base transducer for the fabrication of a uric acid biosensor. Cellulose acetate was deposited onto modified transducer and transducer was coated with uricase enzyme. A polycarbonate membrane was placed onto the resulting electrode to form the sandwich biosensor [80]. In this study enzymatically produced H_2O_2 reacts with the oxidized form of cobalt phthalocyanine to produce the reduced form. The reduced cobalt phthalocyanine is reoxidized on the electron surface. Uric acid determination based on H_2O_2 which can easily pass through the membrane prevented the interferences and direct oxidation of uric acid. Although, screen printed carbon electrode based uric acid biosensors have a lot of advantages such as disposability, portability, easy fabrication, they do not show very low detection limits. More efforts should be done to improve the detection limits.

3.7. Langmuir–Blodgett film based electrodes

Langmuir–Blodgett (LB) films can be described as highly organized ultra thin organic films, ranging from one to several tens of molecular layers. In LB technique a thick Langmuir monolayer is formed at the air–water interface. Langmuir monolayer is transferred onto a solid substrate and multilayer films are fabricated on this solid substrate by the successive deposition of the monolayers [145]. Langmuir–Blodgett (LB) films have the advantages of controllable thickness and minimized loss of biomolecule activity [104,146]. Various biosensors based on Langmuir–Blodgett (LB) films have been developed [146–148]. However, the application of LB films in the area of uric acid biosensors is limited [103,149]. Langmuir–Blodgett technique was used to immobilize uricase enzyme on chitosan/Prussian blue prefunctionalized indium–tin oxide electrode for uric acid biosensor construction [103]. Another study reports the adsorption of uricase onto Langmuir monolayers of stearic acid and the transfer of monolayers to solid supports as Langmuir–Blodgett (LB) films [149]. As LB films show promise for the development of biosensors, more emphasis should be given to this technique in uric acid biosensors.

Table 3 presents a detailed comparison of significant characteristics of various uric acid biosensors based on modified electrodes.

4. Uric acid biosensors based on different modes of transducing devices

Yu et al. [150] reported a chemiluminescence biosensor based on a microfluidic paper-based analytical device. The sensing principle of the biosensor is the chemiluminescence reaction between rhodamine derivative and enzymatically generated H_2O_2. Chu et al. [151] reported an electrochemiluminescent biosensor for uric acid detection with luminol as a signaler. The uricase was immobilized in polypyrrole matrix on platinum electrode during electropolymerization. The biosensor is based on the electrochemiluminescent intensity of luminol increased by the enzymatically produced H_2O_2. The biosensor shows linear response to uric acid from $7.5\times10^{-11}-8.3\times10^{-6}$ M with a detection limit of 75 pM. Another electrochemiluminescent biosensor for uric acid was fabricated based on gold screen-printed cells. Luminol–3,3′,5,5′-tetramethylbenzidine (TMB) copolymer was prepared by cyclic voltammetry on gold screen-printed cells and chitosan–uricase mixture was dropped onto the working electrode. The uric acid response of the biosensor is linear between 1.5×10^{-6} and 1.0×10^{-4} M and the detection limit is 4.4×10^{-7} M. Interference effect of urea and ascorbic acid was reported; however this effect was eliminated by the dilution of the samples [152].

Martinez-Pérez et al. [153] developed a reagentless fluorescent sol–gel biosensor for uric acid detection. The sol–gel was prepared by the encapsulation of uricase, horseradish peroxidase and amplex red. H_2O_2 produced by the enzymatic reaction of uricase leads to the oxidation of amplex red to flourescent resorufin in the presence of horseradish peroxidase. Biosensor response was reported to be linear up to 1 μM with a detection limit of 20 nM. The effect of interferences was eliminated by the dilution of the samples. Schrenkhammer and Wolfbeis [154] reported an optical uric acid biosensor. Ruthenium-based oxygen sensitive beads and iridium-based oxygen sensitive beads was prepared and used as fluorescent probe for oxygen. Glutaraldehyde was used for crosslinking of uricase. Ruthenium-based oxygen sensitive beads or iridium-based oxygen sensitive beads were dispersed in a solution of polyurethane hydrogel; crosslinked uricase was immobilized into the hydrogel to fabricate two types of biosensors. The response of the biosensor is based on the consumption of oxygen during the enzyme catalyzed oxidation of uric acid. Iridium based biosensor showed linear response to uric acid between 5 and 600 μM with a detection limit of 2 μM and the response time was reported as 1.3 min. Ruthenium based biosensor showed linear response to uric acid between 5 and 800 μM with a detection limit of 5 μM and the response time was reported as 1.2 min.

A potentiometric uric acid biosensor based on immobilization of uricase onto zinc oxide nanowires grown on a gold coated flexible plastic electrode was fabricated [155]. The response of the potentiometric biosensor was linear from 1 μM to 650 μM with a detection limit of 1 μM. No significant effect of the common interferences was reported. When the same enzyme electrode was covered with nafion membrane the linear working range was found to be 1–1000 μM. However, the response time increased from 6.25 s to 9 s with the use of the membrane. Wu et al. [156] developed a pH sensitive electrolyte–insulator–semiconductor

Table 3
Characteristics of uric acid biosensors based on modified electrodes.

Enzyme, immobilization technique and matrix used	Uricase source	Detection limit	Linear range	Sensitivity	Stability	Response time	Applications	Ref.
UOX, doping and undoping of the polymer, polyaniline film	*Aspergillus niger*	–	0.0036–1.0 mmol/dm^3	–	18% loss after 60 days	–	–	[67]
UOX, doping and undoping of the polymer, polyaniline film	*Aspergillus niger*	–	1.0×10^{-3}–1.0 mmol dm^{-3}	–	$<$50% loss after 50 days	–	–	[82]
UOX, glutaraldehyte cross-linking, polyaniline film	*Bacillus fastidious*	0.01 mM	0.01–0.05 mM 0.1–0.6 mM	47.2 mA mM^{-1} 10.66 mA mM^{-1}	5% loss after 17–18 weeks	60 s	Uric acid in serum	[117]
UOX, doping and undoping of the polymer, polyaniline film	Pig liver	–	0.001–0.5 mmol dm^{-3}	–	19% loss after 50 days	–	–	[118]
UOX, glutaraldehyte cross-linking, polyaniline-polypyrrole film	Microorganism	1.0×10^{-6} M	$2.5\times10^{-6}-8.5\times10^{-5}$ M	–	20% loss after 4 weeks	70 s	–	[120]
UOX, layer by layer, poly(ethylene imine) (PEI) or poly(diallyldimethylammoniumchloride) (PDAC) films	*Bacillus fastidiosus*	0.15 μA μmol l^{-1} cm^{-2}	0.1–0.6 μM	–	30–40% loss after 30 days	2 s (PDAC) and 6 s (PEI)	–	[121]
UOX, mixed with chitosan solution, hemoglobin encapsulated chitosan matrix	–	0.85 μM	2.0–30 μM	29.5 μA mM^{-1}	11% loss after 20 days	60 s	Uric acid in urine	[123]
UOX, glutaraldehyte cross-linking, 1,4-benzoquinone modified carbon paste	*Arthrobacter globiformis*	1.9×10^{-8} M	$1.9\times10^{-8}-2.8\times10^{-7}$ M $4.8\times10^{-6}-2.8\times10^{-5}$ M $3.5\times10^{-5}-4.9\times10^{-4}$ M $6.3\times10^{-4}-2.7\times10^{-3}$ M	–	15% loss after 15 days	150 s	Uric acid in serum	[125]
UOX, glutaraldehyte cross-linking, poly(vinylferrocenium) modified carbon paste	*Arthrobacter globiformis*	7.4×10^{-8} M	$7.4\times10^{-8}-2.8\times10^{-7}$ M $5.0\times10^{-6}-9.0\times10^{-5}$ M $3.0\times10^{-4}-7.0\times10^{-3}$ M	–	68% loss after 30 days	120 s	Uric acid in serum	[125]
UOX, electrostatic binding, ZnO film	*Arthrobacter globiformis*	2.0×10^{-6} M	5.0×10^{-6}–1.0×10^{-3} M	–	19.5% loss after 20 days	–	Uric acid in urine	[134]
UOX, drop coating, SnO_2 modified multiwalled carbon nanotube	*Arthrobacter globiformis*	5.0×10^{-8} mol L^{-1}	$1.0\times10^{-7}-5.0\times10^{-4}$ mol L^{-1}	–	20.5% loss after 20 days	–	Uric acid in rat striatum	[129]
UOX, carbodiimide linkage, multiwalled carbon nanotube–gold nanoparticle composite	–	0.01 mM	0.01–0.8 mM	–	45% loss after 4 months	7 s	Uric acid in serum	[130]
UOX, chitosan–glutaraldehyde crosslinking, Prussian blue nanoparticles, carboxylated multiwalled carbon nanotube, polyaniline, gold composite	–	5 μM	0.005–0.8 mM	–	37% loss after 7 months	4 s	Uric acid in serum	[131]
UOX, electrostatic binding, ZnO nanoparticles modified multiwalled carbon nanotubes	*Arthrobacter globiformis*	2.0 mM	5.0 μM–1 mM	393 mA cm^{-2} M^{-1}	11% loss after 160 days	–	Uric acid in urine	[132]
UOX, drop casting, MPx-11 modified mixed self-assembled monolayer of 2-(2-mercaptoethylpyrazine) and 4,4′-dithiodibutyric acid	*Candida sp.*	2×10^{-6} M	5×10^{-6}– 1.5×10^{-4} M	3.4 ± 0.08 nA cm-2μM-1	–	10 s (low conc.) 80–100 s (high conc.)	–	[139]
UOX, Bis[sulfosuccinimidyl] suberate (BS3) cross-linking, self-assembled monolayer (SAM) of 3-aminopropyltriethoxysilane (APTES)	*Bacillus fastidiosus*	0.037 mM	0.05–0.58 mM	39.35 μAmM+	10% loss after 7 weeks	50 s	–	[140]
UOX, glutaraldehyde crosslinking, poly(N-methyl-o-phenylenediamine)modified self-assembled monolayer of 2-aminoethanethiolate	*Candida sp.*	–	0.15–0.40 mmol dm^{-3}	0.066 μA cm^{-2}	–	30 s	–	[141]
UOX, enzyme was dissolved in the suspension of L-α-phosphatidylcholine β-oleoyl-γ-palmitoyl (PCOP) liposomes.	*Candida sp.*	–	–	0.95 ± 0.01 μA cm^{-2}	–	20 s	–	[142]
UOX, enzyme was coated on cobalt phthalocyanine modified screen printed carbon electrode	*Arthrobacter globiformis*	1.3×10^{-5} M	1.3×10^{-5}–6×10^{-3} M	–	14% loss after 7 days	44 s	Uric acid in urine	[144]
UOX, casting method, Ir-modified carbon ink	*Bacillus fastidiosus*	0.01 mM	0.1–0.8 mM	16.60 μAmM^{-1}	–	24.7–40.8 s	–	[81]
UOX, cellulose acetate deposited screen-printed carbon electrode pre-modified with electrocatalyst cobalt phthalocyanine	*Arthrobacter globiformis*	0.015 mM	0.015–0.25 mM	2.10 μA/mM	–	–	Uric acid in urine	[80]

sensor with a layer of $Sm2TiO_5$ for uric acid detection. Uricase was immobilized into alginate hydrogel and this layer was attached to the electrolyte–insulator–semiconductor sensor. The detection mechanism of the sensor is based on the pH changes due to the reduction of enzymatically formed H_2O_2. Uricase was immobilized into stabilized lipid films using zinc oxide nanowires to fabricate a potentiometric uric acid biosensor [157]. The biosensor response was reported to be linear between 1 and 1000 μM with a detection limit of 0.4 μM and the response time was 6 s. Ascorbic acid, glucose, urea, proteins and lipids showed no interference effect. Liao et al. [158] prepared a potentiometric uric acid biosensor by the immobilization of catalase, uricase and ferrocenecarboxylic acid (mediator) on the surface of SnO_2–indium tin oxide glass. 3-Glycidyloxypropyltrimethoxysilane was used to activate the SnO_2 film surface and form a convenient matrix for enzyme and mediator immobilization. The biosensor showed linear response to uric acid between 2 and 7 mg/dL with a response time of 3.5 minutes. No significant interference effect of ascorbic acid, glucose and urea was reported.

Mulyasuryani and Srihardiastutie [159] developed a conductometric uric acid biosensor. Nata de coco (fermantation product from coconut water by Acetobacter xylinum) membrane was prepared from coconut water, sugar, acetic acid and Acetobacter xylinum. Uricase was immobilized on a nata de coco membrane-Pt electrode. The biosensor response to uric acid was linear between 1 and 6 ppm. The response time of the biosensor was reported to be three minutes. The biosensor was used for uric acid determination in human serum samples. A conductometric single ZnO nanowire based field-effect transistor (FET) biosensor was developed for uric acid determination [164]. Single ZnO nanowires were synthesized by the chemical vapor deposition method. ZnO and carbon power were mixed and transferred into an Al_2O_3 boat inside a quartz tube. This reaction system was kept at 980 °C under argon (carrier gas) and oxygen (reaction gas) flow for 20 minutes for ZnO nanowire formation. Uricase was covalently immobilized to the surface of the biosensor. The detection of the biosensor was reported as 1 pM.

5. Conclusions and future trends

Uric acid is a primary end product of purine metabolism in humans. Abnormal uric acid levels in biological fluids are symptoms of several conditions. Therefore, the demand for the routine analysis of uric acid is increasing, and simple, rapid and low-cost methods for its determination are necessary. Electrochemical sensors based on the oxidation of uric acid seem to be a good approach for uric acid determination. However, these sensors generally suffer from varying degrees of interference from ascorbic acid or dopamine which can be oxidized at a potential close to that of uric acid. Incorparation of enzymes in electrochemical sensors can be a solution to this problem. Uric acid can be efficiently determined using amperometric biosensors. In this review recent work in the development of amperometric uric acid biosensors with an emphasis on the detection mechanisms, types of immobilization techniques and the different types of electrodes used are described in detail. The comparative characteristics of the various uric acid biosensors have been summarized in the tables.

In the market commercial uric acid meters such as Benechek Plus meter [160], Multisure uric acid meter [161], Easytouch uric acid monitoring system [162] and uric acid test kits [163] are available. In commercial meters the single use strips and sterile lanchets increase the cost of the analysis and the analysis time with the test kit is not very short (30 min). Moreover, the cost of the enzymes used in the kit limits the practical use. On the other hand, in biosensor technology, the immobilization of enzymes on a convenient substrate reduces the cost of the procedure. For practical applications of uric acid biosensors, problems like interference, reproducibility, stability and real sample measurements present the major limitations. Hence, the development of cheap, rapid, accurate and disposable biosensors for the detection of uric acid is still needed. At this point, screen-printed electrodes offer without doubt an important approach toward the development of disposable, selective, sensitive uric acid biosensors for routine analysis. Recent developments in microelectrode fabrication and screen printing technology suggest that future efforts could yield low cost, commercially available, screen-printed electrode based uric acid biosensors. Electrochemical biosensors, based on nanomaterials (carbon nanotubes, nanorods, nanometal oxides etc.) also offer great promise for uric acid biosensor development. We believe that improvements in nanomaterials and nanofabrication technologies will also lead to the fabrication of small, low cost, portable and disposable commercial uric acid biosensors. This approach can also be expanded for the construction of biocompatible uric acid biosensors for in vivo measurements.

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