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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.

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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:

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

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

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