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Lignin modified glassy carbon electrode for the electrochemical determination of histamine in human urine and wine samples

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ABSTRACT

Lignin was deposited at the surface of glassy carbon electrode potentiostatically. Cyclic voltammetric investigations revealed the surface modification of glassy carbon electrode by an electroactive polymer film. Lignin modified electrode showed catalytic activity towards the oxidation of histamine in phosphate buffer solution. A limit of detection of 0.28×10^{-6} mol L^{-1} (LoD = 3s/slope) was achieved in the linear concentration range of 5×10^{-6} to 2×10^{-4} mol L^{-1}. Along with the excellent recoveries in the range 92.76-100.20% of spiked histamine from human urine and red wine samples, the interference and stability results showed the potential applicability of the developed method for the determination of histamine in real samples.

1. Introduction

Alkaloids represent a group of natural products that have a major impact throughout history on the economic, medical, political and social affairs of humans. Many of these compounds have potent physiological effects on mammalian systems as well as other organisms, and as a consequence, some constitute important therapeutic agents [1]. Alkaloids are organic compounds known for their medicinal and, paradoxically, poisonous attributes [2–4].

Histamine (Fig. 1) is an alkaloid which is enzymatically produced by the decarboxylation of the corresponding amino acid, histidine, in the presence of the microbial decarboxylase enzyme. It is also produced by bacterial action during food processing and storage and hence could be present in substantial amounts in fermented foodstuffs and beverages. The histamine content in food is commonly used as an indicator of food deterioration, and frequently regarded as one of the biomarkers in quality control monitoring during food production, storage and transportation [5].

Both histamine deficiency and excessive histamine may cause neurological and physical disorders. One possible cause for histamine deficiency is the presence of excess copper levels, as it decreases blood histamine. Histamine ingested from a food at a level of more than 2.7 mg/kg body weight induces symptoms of histamine intolerance. An adverse reaction is also experienced by a few sensitive individuals on ingestion of lower concentrations of histamine. Histamine at levels usually exceeding 1000 mg/kg has been implicated with certain food intoxications such as the cheese syndrome [6,7].

Histamine has been assumed to contribute to embryo–uterine interactions due to its vasoactive, differentiation and growth-promoting properties. However, its exact functions in pregnancy are unclear. The histamine-degrading enzyme, diamine-oxidase, is produced in high amounts by the placenta and has been supposed to act as a metabolic barrier to prevent excessive entry of bioactive histamine from the placenta into the maternal or fetal circulation. Biogenic amines such as histamine, tyramine, and phenyl ethylamine are known to induce nausea, headaches, and respiratory disorders in sensitive individuals, particularly when accompanied by alcohol and acetaldehyde intakes. These amines are normally metabolized by amine-oxidases to keep their steady-state concentrations low. However, for individuals with reduced monoamine-oxidase (MAO) activity and individuals taking MAO inhibitors, ingestion of foods containing large amounts of tyramine can lead to transient hypertension, hypertensive crisis, and panic attacks [8–10]. For such indigenous compounds of high physiological activity, the synthesis, transport, storage, release and degradation are regulated by complex biochemical processes. Hence, there is a considerable interest in the development of sensitive analytical methods for the determination of histamine in real samples.

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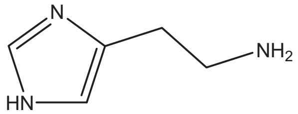

Fig. 1. Chemical structure of histamine.

Capillary zone electrophoresis [11], micellar electro-kinetic chromatography [12], thin-layer chromatography [13] and high-pressure liquid chromatography [14] are among the common methods reported on the determination of histamine. However, the reported conventional methods are economically expensive, need trained personnels and use organic solvents making them environmentally non-friendly. Therefore, an electrochemical method which is economical, more sensitive and environmentally friendly is needed to determine the amount of histamine present in real samples.

Few works [5,6,15–17] have been reported on the electrochemical determination of histamine. However, the electrode modifiers such as gold and Nafion they used are so expensive besides the complexity of the modification procedures. Thus, an electrochemical method using a cheap and easily available material as electrode modifier should be developed.

Lignin is a natural amorphous polymer contained in most woody plants such as trees and shrubs. As sited elsewhere [18], approximately 2% of the lignin released during chemical pulping in pulp and paper industries is used for chemical and material applications and the remainder is burned as boiler fuel showing its availability. The electrochemical behavior of lignin at the surface of electrode material has also been reported [19]. To the best of our knowledge, voltammetric determination of histamine using lignin modified glassy carbon electrode has not been reported.

2. Experimental

2.1. Instruments and chemicals

Voltammetric experiments were conducted using CHI760D Electrochemical Workstation, CH Instruments (Austin, Texas, USA). All electrochemical experiments were carried out in a conventional three-electrode system with a glassy carbon electrode (3 mm in diameter) or lignin modified glassy carbon electrode as a working electrode, platinum wire as an auxiliary electrode and Ag/AgCl electrode as a reference electrode. All experiments were conducted at $20 \pm 2\,^{\circ}C$. Histamine and alkali lignin were supplied by Sigma Aldrich (USA). All other reagents were of analytical grade and used directly without purification. Distilled water was obtained by purification through a Millipore water system and used throughout.

2.2. Analytical procedure

A 10 mM histamine stock solution was prepared using (pH 7) 0.1 M phosphate buffer solution (PBS). Working solutions of histamine were prepared from the stock solution by series dilution. Conversely, alkali lignin was prepared by dissolving 10 mg lignin in every 1 mL 0.1 M HNO_3. The prepared lignin solution was then mixed with 0.5 M H_2SO_4 used as a supporting electrolyte in a 1:1 volume ratio. Lignin polymer film was deposited at the surface of glassy carbon electrode following the procedure under section 2.3. After each voltammetric measurement using the polymer modified electrode, the surface of the electrode was renewed by running linear sweep in the opposite potential scan direction.

Cyclic voltammograms of the unmodified and polymer modified glassy carbon electrodes in the absence and presence of histamine were recorded in the potential range 0 to +1.6 V. After the optimization of the solution and square wave voltammetric parameters, a calibration curve was obtained using series of standard histamine solutions.

2.3. Preparation of lignin modified glassy carbon electrodes

The lignin modified glassy carbon electrode was prepared following a procedure reported elsewhere [19] with minor modification. Briefly: A glassy carbon electrode (3 mm diameter) was first rinsed with distilled water, polished carefully with alumina powder having different particle size (1.0, 0.3 and 0.05 μm) to a mirror finish surface. The residual polishing material was removed by repetitive rinsing of the surface with distilled water. The resulting electrode was immersed in pH 7 PBS, and was activated potentiodynamically by scanning the potential between -0.2 and +1.5 V for five cycles at a scan rate of 100 mV s^{-1}. Then, the activated electrode was transferred to a cell containing a mixture of 0.5 M H_2SO_4 and 0.1 M HNO_3 containing 10 mg/mL lignin mixed in 1:1 volume ratio. Lignin polymer was then deposited at the electrode surface potentiostatically by applying a potential of +0.9 V for 2 minute. The lignin modified electrode was then rinsed with distilled water to remove physically adsorbed and unreacted species from the electrode surface. Subsequently, the modified electrode was stabilized in pH 7 PBS by scanning the potential between −0.2 V and +1.0 V until a steady cyclic voltammogram was obtained. Finally, the modified electrode was dried in air and made ready for use.

2.4. Real sample preparation

Commercial red wine purchased from a super market and human urine collected from a volunteer were both suction filtered. The filtrate collected for each sample (wine and urine) was first diluted with pH 5.5 PBS in a volume ratio of 1:20 (filtrate:buffer). Then, three concentrations of 40, 60, and 80 μM histamine solutions were prepared using the buffer diluted commercial red wine and human urine filtrates separately. The 40, 60 and 80 μM standard histamine in the red wine and human urine samples were used to study the applicability of the developed method for the determination of histamine in real samples.

3. Results and Discussion

3.1. Potentiostatic deposition of lignin at the surface of GCE

As briefly discussed under the experimental section, lignin was deposited at the surface of glassy carbon electrode potentiostatically. Fig. 2 presents the steady state cyclic voltammograms of unmodified GCE (A) and lignin modified GCE (B) in 0.1 M HNO_3. The voltammogram of the lignin modified electrode exhibited distinct redox couples (peaks *b* and *b*’ of curve B) which are absent at the unmodified electrode showing the deposition of an electroactive polymer film of lignin at the surface of GCE.

3.2. Effect of scan rate on lignin modified glassy carbon electrode

The effect of scan rate was used to further verify the deposition of lignin film at the surface of GCE. Fig. 3A shows the cyclic voltammograms of lignin modified GCE at different scan rates. As can be observed from the figure, the peak potentials for the redox couple (peaks *b* and *b*’) are independent of the potential scan rate indicating the reversibility of the redox couples. Furthermore, the peak current response for the oxidative peak (peak *b*’ of Fig. 3B) showed linear dependence on the scan rate signifying that the redox couples are due to a surface confined process which is still confirmation for the deposition of lignin film at the electrode surface [20].