Effective Determination Of Homovanillic Acid Using A Poly (4-Amino-3-Hydroxy) Naphthalene 1-Sulphonic Acid Modified Glassy Carbon Electrode

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Abstract:

Homovanillic acid (HVA) is a major dopamine metabolite associated with dopamine levels in biological system. For the analysis of HVA, a sensor based on electrochemical oxidation of poly (4-amino-3-hydroxy) naphthalene 1-sulphonic acid was developed. The sensor showed wide linear range from 2 x 10^{-4} M to 2 x 10^{-6} M with the detection limit of 7 x 10^{-7} M using differential pulse voltammetry. The kinetics and mechanism of the electrochemical process was studied by Laviron model and the utility of the sensor was assessed by quantifying HVA in artificial physiological fluid samples.

Keywords: HVA, poly (4-amino-3-hydroxy) naphthalene 1-sulphonic acid, voltammetry, electrocatalytic oxidation, electrochemical sensor

Introduction

Developing sensors for the effective determination of biomolecules which can act as biomarkers for various diseases play a pivotal role in clinical analysis. It helps us to carry out investigations on changes in usual metabolic pathways by finding out the abnormalities in the preferred level of such biomolecules. In this scenario, electrochemical techniques find importance because of their renowned properties like simplicity and robustness in sensitive and reliable determination of various analytes and thus exclusively replaces other well known chromatographic methods.[1] In addition to this, electrochemical techniques explore the possibility of using crude samples without much pretreatment and thus lowering matrix effects for the analysis of electroactive species. This article shed light on an electrochemical sensing platform for the major catecholamine metabolite Homovanillic acid (HVA) on polymer modified glassy carbon electrode (GCE).

Homovanillic acid is a major dopamine metabolite that associated with the dopamine levels in biological system. The normal level of HVA is 0 – 46 µg/mg of urinary creatinine/24 h.[2] An elevated HVA level can be associated with the deficiency of dopamine beta-hydrolase or neuroblastoma or pheochromocytoma hence HVA is regarded as the biomarker for neuroblastoma. Patients suffering from Parkinson disease have been identified with reduced levels of HVA in cerebrospinal fluid (CSF) and blood plasma.[3]

Electrodes that are modified using electropolymers offer highly sensitive and selective sensing platforms for electroactive biomolecules.[4] Being an important biomolecule, several electrochemical sensors are developed for the determination of HVA on carbon based electrodes. This article briefly explains electrooxidation of HVA on poly (4-amino-3-hydroxynaphthalene-1-sulphonic acid) modified glassy carbon electrode ((poly 4A3HNSA)/GCE). Application study was carried out in artificial physiological fluid samples.

Experimental

Reagents: All the chemicals were of analytical-reagent grade and used without further purification. 4A3HNSA was obtained from Sigma Aldrich, India. HVA was procured from Alpha Aesar, India. Stock solution of HVA (10^{-2} M) was prepared by dissolving the 18.22 mg of the compound in ethanol. Working standard solutions were prepared by serial dilution of freshly prepared stock solution as and when required. All other solutions were prepared in double distilled water.

Apparatus: Electrochemical studies were carried out using an electrochemical work station (CH Instruments, USA). A three-electrode system, in which Ag/AgCl as the reference, Pt wire as the counter and poly 4A3HNSA modified GCE as the working electrode, was used . Before each measurement, the solution in the cell was purged with nitrogen and measurements were made under unstirred condition at room temperature. Scanning Electron Microscopic (SEM) images were obtained on a JOEL 6390LV. pH measurements were carried out in a Metrohm pH meter. HPLC analysis was done using HPLC-UV (LC-20AT, SPD-20A, SHIMADSU).

Fabrication of poly 4A3HNSA modified GCE: Before the modification, GCE was polished with alumina slurry (0.05 micron) in a polishing pad and sonicated sequentially in methanol, 1:1 HNO_3 solution, acetone and water for 5 minutes respectively. Electropolymerisation of poly 4A3HNSA on GCE was done by cyclic potential scanning from -0.5 V to 2.0 V for 10 cycles at a scan rate of $0.1Vs^{-1}$. Polymer modified electrode was then activated by cyclic scanning in the potential window -0.75 V to 0.65 V for 6 cycles.[5]

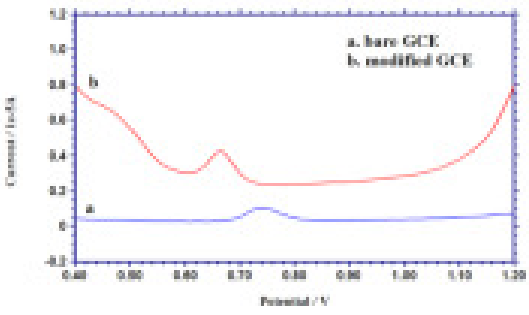
Experimental procedure: Prior to voltammetric measurements, the working solution was degassed with N_2. Studies were done in conventional electrochemical cell containing suitable amount of HVA in 10 mL of 0.1 M PBS (pH = 3) using Differential Pulse Voltammetric (DPV) technique. Potential scanning was done from 0.4 V to 1.2 V.

Measurement of HVA in artificial biological samples: Artificial biological fluids were prepared according to reported procedures.[1,6]

Results and Discussion

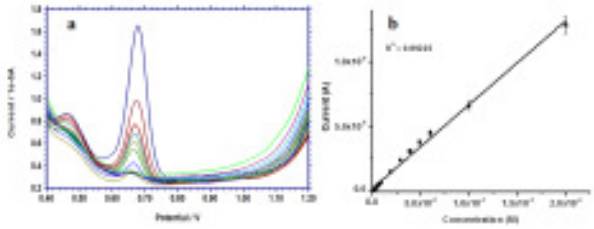
Optimisation of experimental conditions: Among the various supporting electrolytes studied (sulphuric acid, sodium chloride, potassium chloride, acetate buffer, citrate buffer, phosphate buffer, sodium hydroxide and potassium hydroxide) the lowest peak potentials were obtained with 0.1 M PBS. Hence further electrochemical studies of HVA were carried out using 0.1 M PBS as the supporting electrolyte. The polymer film grown by 10 cycles of polymerisation gave the best current response for the electro-oxidation processes and hence all the experiments were performed in the electrode fabricated by 10 polymerisation cycles.

Voltammetric behaviour of HVA on the poly (4A3HNSA)/GCE): On the *p*4A3HNSA/GCE, well defined and well separated peaks were obtained for the oxidation of HVA, using 0.1 M PBS as supporting electrolyte. Compared to the bare GCE, the oxidation of the HVA on *p*4A3HNSA/GCE took place at a lower potential with an enhanced peak current as can be seen from Fig. 1.



Under optimized conditions, the sensor was found to be useful for the quantification of HVA in the range 2.00×10^{-4} M to 2.00×10^{-6} M. [16]The limit of detection (LOD) was found to be 7.00×10^{-7} M.

Figure2. (a) Overlay of DPVs recorded in 0.1M PBS pH 3 containing varying amount of HVA in the range 2.00×10^{-4} M – 2.00×10^{-6} M (b) Linear correlation diagram between peak current and concentration of HVA



Interference:

Effect of various co-existing species on the electrochemical oxidation of HVA on poly 4A3HNSA modified GCE electrode was investigated by measuring the variation in current response of 4.00×10^{-5} M HVA in the presence of different species at 1:1, 1:10 and 1:100 molar concentration ratios with the analyte. It was found that ascorbic acid, adrenaline and uric acid do not interfere the analysis up to 1:1 molar ratio with HVA. L-dopa, norepinephrine and dopamine interfere the analysis even when present at 1:1 molar concentration with HVA. The system showed tolerance up to 10-fold excess concentration of creatine. NaCl and creatinine do not interfere even at 100-fold excess concentration as that of HVA.

The validity of the sensor was evaluated by quantifying the amount of HVA in spiked artificial urine samples using the developed method and reported HPLC-UV method[7]

Conclusion: An electrochemical sensor has been developed for HVA based on poly 4A3HNSA modified GCE. The sensor showed a wide linear range from 2.00×10^{-4} M – 2.00×10^{-6} M with a detection limit of 7.00×10^{-7} M. Better electrocatalytic nature of the polymer film enabled sensitive determination of the analyte. Kinetics and mechanism of the electrochemical process was studied by Laviron model. Diffusion coefficient of HVA was evaluated by chronocoulometric technique. Developed sensor was successfully employed for the determination of HVA in artificial physiological fluid samples.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Table.I Application study

Sample	Spiked (M)	Found (M)	Recovery (%)	RSD(%) (n=4)
Artificial Urine	1.00×10^{-4} -2.00×10^{-6}	9.91×10^{-5} - 2.06×10^{-6}	100	1.8
Artificial Cerebrospinal Fluid	6.00×10^{-5} - 2.00×10^{-6}	6.01×10^{-5} - 2.07×10^{-6}	101	2.2
Artificial Serum/ Ringer's solution	8.00×10^{-5} - 2.00×10^{-6}	8.02×10^{-5} - 2.02×10^{-6}	101	0.5