Electroctrochemical Determination of Guanine Using L-Histidine Modified Glassy Carbon Electrode

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Abstract:

Guanine, a purine base found in DNA is a main component of guanosine tri-phosphate. The oxidative damage of DNA can be detected by analysing the level of guanine in urine. Hence in this study, a sensitive voltammetric method for the determination of guanine was developed using a glassy carbon electrode modified with poly-L-histidine (poly (L-His)/GCE). The experimental procedures and conditions for the determination of guanine were studied and optimized. The lowest potential for the electro-oxidation of guanine was obtained in 0.1 M NaOH by square wave voltammetry (SWV). The electrocatalytic oxidation current was found to be linear in the range from 1 μM to 100 μM. The developed poly (L-His)/GCE sensor is simple in preparation and showed excellent selectivity, reproducibility and was successfully applied for the determination of guanine in artificial urine and blood serum.

Keywords: guanine, poly-L-histidine, voltammetry, electrocatalytic, sensor

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Guanine is an important component found in deoxyribonucleicacid (DNA).It can be readily oxidised to 8-oxoguanine which can change its base pairing properties. 8-oxoguanine residues from DNA can pair with adenine and can lead to transverse mutation. The poly-L-Histidine contain imidazole group which has the ability to immobilize biomolecules. The guanine component of the physiological fluids, tissues and cells is related to the catabolism of nucleic acids. Hence the electrochemical detection of guanine is important.

Experimental

Reagents- L-Histidine was purchased from Spectrochem Pvt. Ltd. Mumbai, India. Guanine and xanthine were supplied by s.d fine chemicals. Adenine is supplied by Himedia laboratories Pvt.Ltd. Uric acid and ascorbic acid was purchased from SRL Chemicals, India. All chemicals were of analytical grade and used without further purification. All aqueous solution were prepared in Millipore water .

Apparatus -Electrochemical measurements were performed with an electrochemical work station (CH instruments Austin, TX) controlled by apersonal computer. A conventional three electrode system was used with GCE (bare and modified) as working electrode-saturated Ag/AgCl electrode as reference electrode and counter electrode was a platinum wire. Scanning electron microscope images were obtained on JEOL 6390 LV.

Fabrication of poly-L-histidine P-(L-His)/GCE modified GCE - Prior to use, the GCE was polished with 0.05_M alumina slurry on felt pads, followed by sequential cleaning in ultrasonic bath using Millipore water, methanol, 1:1 HNO_3, acetone and water.

Briefly cleaned GCE, reference lectrode and auxiliary electrode were dipped in a solution of 2.5×10^{-3} M L-histidine. A required number of cyclic potential scanning was applied within the potential range -1.2V to 2.0V at a scan rate of 0.1

V/s to obtain a polymer film of required thickness on the electrode surface. Modified electrode was washed with double distilled water and dried in air. The electrode was activated by Square wave scanning in the potential range 0V to 0.8V in 0.1 M NaOH for 10 cycles.

Results and Discussion

Characterization of p-(L-His)/GCE

Comparison of bare GCE and poly-L-histidine modified GCE points to the change in the surface morphology of GCE, which confirms effective modification of L-His on the surface of bare GCE.

Optimisation of experimental parameters

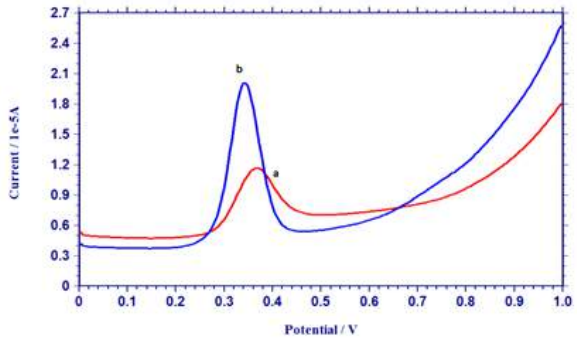
Effect of various supporting electrolyte

The electrochemical properties $1x10^{-4}$ M Guanine in various medium such as PBS, H_2SO_4, KCl, NaOH,Acetate buffer solution and Citrate Buffer solution of 0.1M concentration was investigated by square wave voltammetry. It was observed that lower peak potential and well-defined peak shape was obtained when 0.1 M NaOH was used as the supporting electrolyte.

Electrocatalytic action of L-Histidine on the electrochemical behaviour of guanine

The electrochemical behaviour of $1x10^{-4}$ M guanine showed a decrease in peak potential and more than 3 fold enhancement in peak current. (Fig.1)

Fig1: Square wave voltammogram for the oxidation of Guanine on (a) bare GCE (b) modified GCE



The oxidation peak current of Guanine varies linearly with its concentration in the range of $5x10^{-5}$ to $1x10^{-6}$ .The calibration curve of oxidation peak current versus concentration of Guanine is represented in Fig. 2. From the equation 3S/m, were S=standard deviation and m=slope, the detection limit for the sensor was found to be $1.3487x10^{-6}$ M.

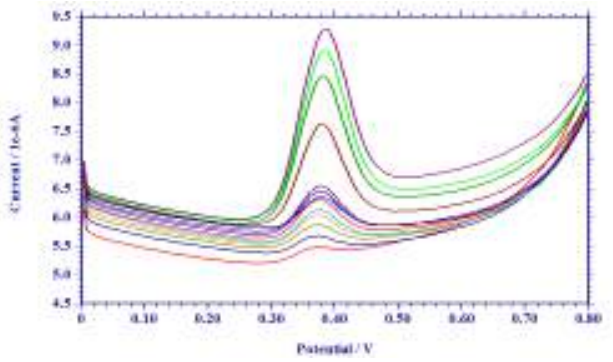


Fig2: Overlay of SWV for the oxidation of $5x10^{-5}$ to $1x10^{-6}$ concentrations of Guanine and the corresponding calibration plot

Interference Study

To determine the influence of foreign species on the determination of Guanine on p(L-His)/GCE some common co-existing species were added along with Guanine.

Serial No.	Coexisting species Species)	Concentration ratio(Gu:coex.	Signal change
1.	Adenine	1:10	0.7495
2.	Caffeine	1:100	4.2181
3.	Xanthine	1:1	6.195
4.	Theophylline	1:10	2.7103
5.	Uric acid	1:100	1.9225
6.	Ascorbic acid	1:10	0.6428
7.	Dextrose	1:100	0.9813
8.	NaCl	1:100	0.6800
9.	KCl	1:100	2.2315

Analytical application

The developed sensor was evaluated for determination of Guanine in urine and blood serum. Good recoveries and RSDs obtained indicates the possible application of the sensor for real sample analysis(Table 3(a) and 3(b).

Table 3(a) Determination of Guanine in urine sample using p-(L-His)/GCE

Added(M)	Found(M)	Recovery(%)	Relative Standard Deviation
$1x10^{-6}$	1.0585	105.85	
$34x10^{-6}$	4.4808	112.02	
$5\ x10^{-6}$	5.0904	101.808	4.0349
$6\ x10^{-6}$	6.1079	101.7983	
$10\ x10^{-6}$	10.3797	103.797	

Table 3(b) Determination of Guanine in serum sample using p-(L-His)/GCE

Added (M)	Found (M)	Recovery (%)	Relative Standard Deviation
$2x10^{-6}$	$1.9194\ x10^{-6}$	95.97	
$3\ x10^{-6}$	$3.1810\ x10^{-6}$	106.033	
$5\ x10^{-6}$	$4.9451\ x10^{-6}$	98.902	3.9073
$6\ x10^{-6}$	$6.1178\ x10^{-6}$	101.963	
$10\ x10^{-6}$	$10.3684\ x10^{-6}$	103.684	

Conclusion

In the present work, voltammetric determination of guanine using a glassy carbon modified with p-(L-His)/GCE was studied. The electro-catalytic oxidation current was found to be linear in the range from $5x10^{-5}$ to $1x10^{-6}$. The contents of guanine in synthetic urine and serum were measured and the results were satisfactory. Therefore, this simple and reliable method could be proposed to determine guanine in the related physiology process.

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