

Graphene Nanoparticles Modified Carbon Paste Electrode Sensor for The Detection of L-Histidine

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Abstract

Voltammetric L-Histidine (HIS) sensor is being developed with graphene modified carbon paste electrode. The fabrication of graphene nanoparticles modified carbon paste electrode (G-CME) enabled electrochemical determination of Histidine (HIS) using differential pulse voltammetry (DPV) and Chronocoulometry. The graphene nanoparticles showed a synergistic effect by showing enhanced electrochemical of HIS by increasing the peak current with a corresponding decline of peak potential compared to carbon paste electrode which makes the reduction process thermodynamically more favorable. The modified electrode had a good sensitivity towards determination of HIS in a linear working range of $1.59 \times 10^{-7} M$ to $2.60 \times 10^{-3} M$ with corresponding detection limit of $1.26 \times 10^{-8} M$ for HIS

Keywords: Graphene nanoparticles, Histidine, cyclic voltammetry, Differential pulse voltammetry, Chronocoulometry

1. Introduction

Amino acids contain both amino group and carboxylic acid functional group are classified under organic compounds They are fundamental building blocks of protein and second largest component of human muscles crucial for repairing tissues, breaking down food, and synthesizing hormones. Amino acids required for body function like improved muscle growth, increased energy, and metabolic regulation . They are divided into essential aminoacids and nonessential aminoacids . Histidine is an essential amino acid. It is not made by the body, acquired through diet. [1]. Histidine is involved in many different metabolic processes in the body. Metabolism of histidine in the body produces histamine, which is involved in the inflammatory response and the production of hydrochloric acid by the stomach[2]. People use histidine for diarrhea due to cholera infection, eczema, kidney failure, metabolic syndrome, and many other conditions, but there is no good scientific evidence to support these uses. If one of the essential amino acids is less than needed for an individual the utilization of other amino acids will be hindered and thus protein synthesis will be less than what it usually is, even in the presence of adequate total nitrogen intake Protein deficiency has been shown to affect all of the body's organs and many of its systems, including the brain and brain function of infants and young children; the immune system, thus elevating risk of infection; gut mucosal function and permeability, which affects absorption and

vulnerability to systemic disease; and kidney function. The physical signs of protein deficiency include edema, failure to thrive in infants and children, poor musculature, dull skin, and thin and fragile hair. Biochemical changes reflecting protein deficiency include low serum albumin and low serum transferrin [3]. Determination of amino acids is important in various fields of research, particularly in food, soil, biotechnology and pharmaceutical industries.

L-Histidine (HIS) is only produced in very small amounts by the body, it must predominantly be taken in through the diet. It is involved in the formation of proteins and influences several of the metabolic reactions in the body. Infants in particular need an additional source of L-histidine, either through breast milk, special supplements, or formula milk, as a deficiency can lead to growth problems and other conditions. It is an amino acid that is abundant in hemoglobin, the iron-rich protein that carries oxygen to every cell in the human body. HIS is also an essential substance for those recovering from an illness and during growth (in childhood and the teenage years). It is best to take HIS on an empty stomach, as it is then more effective. An unbalanced diet or too much stress can also lead to a deficiency, which can manifest itself in severe metabolic and developmental issues, manifesting as growth retardation, anemia, and eczema. HIS regulates the immune defense in the body, allergic reactions and inflammatory processes, so a deficiency of HIS can lead to an increased tendency towards infection and the aggravation of symptoms of allergies. Histidinemia is a disease when there is excess of histidine in the body due to a metabolic enzyme defect [4].

The development of efficient analytical methodologies for the detection of HIS is important for the identification and treatment of HIS deficiency. Spectroscopic methods have been proposed such as chemiluminescence, fluorescence, spectrometry [5]. In general, one of the main problems of these methodologies is the need for previous derivatization of the amino acids.

The detection of amino acids by direct electrochemical detection on bare electrodes has been the focus of attention by various researchers. Various electroanalytical methods and sensors for the detection of amino acids have been at bare and modified electrodes [6-8]. The use of these electrodes bears the advantage of disposability.

This study aims to determine the detection limit of HIS voltammetrically. The objective of this investigation was to study the electrochemical properties of the histidine using Cyclic Voltammetry, differential pulse voltammeter and Chronocoulometry.

2. Material and Methods

2.1 Chemicals:

All chemicals were of Analytical grade and were used as received without further purification. L-Histidine and graphene powder (size < 0.2nm) were purchased from S.D. fine Chemicals, India. Mineral oil was purchased from Fluka, India. Double distilled water was used for the preparation of aqueous solutions having a specific conductivity 0.4 -0.9 μ S.

2.2. Instrumentation:

All voltammetric measurements and electrochemical impedance spectroscopy (EIS) study have been performed on Eco Chemie, Electrochemical Work Station, model Autolab PGSTAT 30 using GPES software version 4.9005. A three electrode system employing an Ag/AgCl (3M KCl) and

platinum electrode were used as reference and counter electrodes respectively. Graphene nanoparticles modified carbon paste electrode (G-CME) was used as working electrodes. The pH measurements were performed using an ELICO LI 120 pH meter.

2.3. Preparation of carbon paste electrode (CPE) and Graphene nanoparticles modified carbon paste electrode (G-CME)

CPE was prepared by mixing graphite with mineral oil at composition 70:30 (w/w) using a motor and pestle and was allowed to homogenize for 48 hours [8]. Graphene nanoparticles modified carbon paste electrode (G-CME) was prepared by mixing mineral oil, graphite powder and graphene nanoparticles with various weight ratios (Table1). The pastes were then packed into a Teflon micro tip (diameter 0.5mm) and a copper wire inserted into the paste established an electrical contact. A new surface was regenerated by pressing out an excess of paste out of the tip and polishing it against zero grade butter paper until the surface had a shiny appearance.

Electrode	Graphite (mg)	Graphene nanoparticles (mg)	Mineral oil (mg)
G-CME -1	60	10	30
G-CME - 2	60	9	30
G-CME - 3	60	8	30
G-CME - 4	60	6	30
G-CME – 5 (G-CME)	60	5	30
G-CME - 6	60	4	30
G-CME - 7	60	2	30

Table 1: Composition of various modified electrode with the weight ratios of graphite: graphene nanoparticles: mineral oil

2.4. Determination of L-Histidine

Differential pulse voltammetric (DPV) studies were carried out with appropriate quantity of the analyte (HIS) in 50mL standard volumetric flask and then making up to the mark with pH 4.0 Phosphate buffer (Phos). The solution was then transferred into an electrochemical cell and the measurements were carried out at $25 \pm 0.2^\circ\text{C}$. N_2 gas purging was not required as oxygen did not interfere in the measurements. DPVs were recorded within the potential range – 0.9 to – 0.1 V with a scan rate of 10 mVs^{-1} and modulation amplitude of 50 mV.

3. RESULTS AND DISCUSSION:

3.1. Effect of pH:

Standard solutions of HIS ($1 \times 10^{-6} \text{M}$) were used to find the optimum pH of the supporting electrolyte at CPE. The influence of pH on the oxidation peak current of HIS was investigated in the pH range of 2 to 10 employing Britton-Robinson (BR) buffer (0.04M) by DPV. The largest peak current was obtained at pH 4 for the analyte HIS (Fig 1) . The negative shift of E_p values with increasing pH indicates the

involvement of proton transfer preceding the potential determining step [9]. The peak currents were found to increase in the beginning with the increase in pH showing maximum at pH 4.0 and decrease thereafter. This could be due to the fact that the reduction became kinetically less favorable due to repulsive electrostatic interactions with the surface of the electrode. Therefore this pH was selected as the optimum pH for further studies.

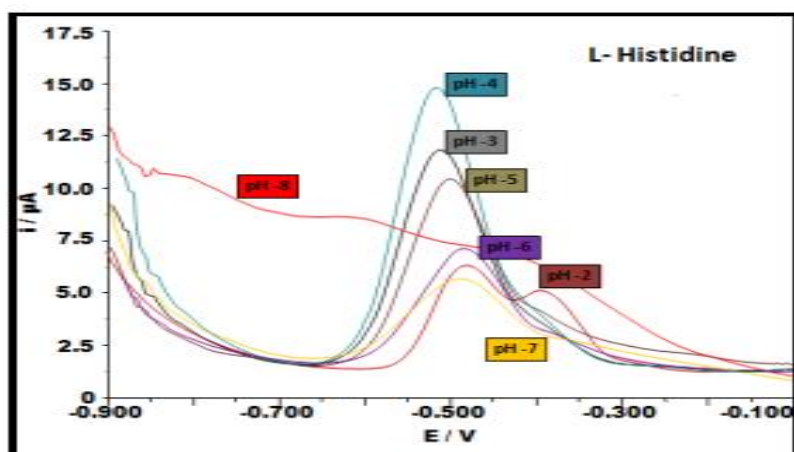


Fig. 1: pH study by Differential pulse voltammetry for reduction of $1 \times 10^{-6}M$ HIS at; CPE vs. Ag/AgCl; in 0.04M BR buffer ; scan rate 100mV/s at 25°C

Equal number of protons and electrons were involved in the reduction. Further various buffers such as Britton-Robinson (BR), Phosphate (Phos), Citrate (Cit), Acetate (Act) and Potassium hydrogen phthalate (KHP) buffer were used at pH 4.0. The concentration of the buffers was taken as 0.1M except for BR buffer where concentration was 0.04M. Amongst all the buffers used, Phosphate buffer gave the best response in terms of peak current and peak shape for both HIS. Thus Phosphate buffer was chosen for further experiments.

3.2. Effect of surface modification and Optimization of the amount of the modifier:

The effect of modifier for the oxidation of HIS was studied using DPV. Fig. 2 represent the DPV's for HIS ($1 \times 10^{-6}M$) at CPE and G-CME. It is observed from this figure that the modification of the CPE with graphene nanoparticles enhanced the HIS signal considerably by about 2.7 times with a concurrent shift in E_p from -0.485 V to -0.0514 V for HIS towards more negative side as compared to CPE. This is due to the synergistic effect where graphene nanoparticles are acting as a promoter by increasing the electro-active surface area.

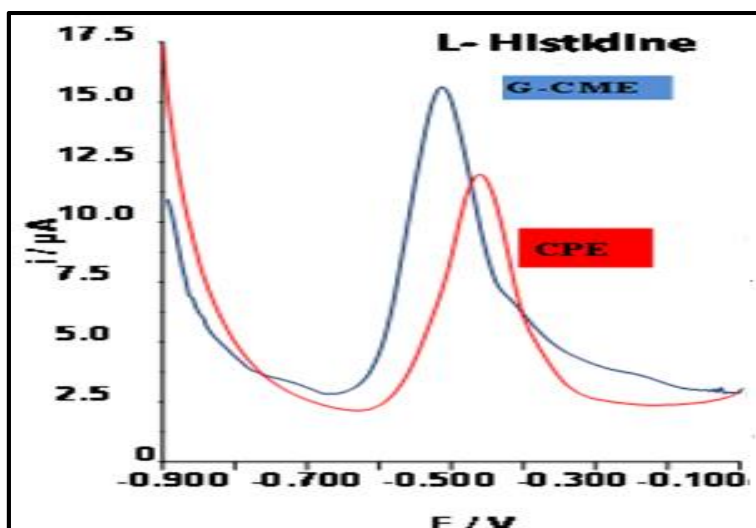


Fig. 2: Differential pulse voltammetry for reduction, of $1 \times 10^{-6} \text{M}$ HIS at CPE and G-CME vs. Ag/AgCl; in 0.1M Phosphate buffer (pH 4); scan rate 100mV/s at 25°C

The electrochemical response in terms of the influence on the amount of graphene nanoparticles added to CPE was studied. The peak current increased with increase in the percentage weight of nanoparticles till the ratio of composition (w/w) for graphite: graphene nanoparticles: mineral oil was 60:5:30. Beyond this composition there was a gradual decrease in peak current. This can be due to the increase in resistance of the electrode to electron transfer. The presence of excessive nanoparticles in the modified electrode may obstruct the diffusion process of the oxidation reaction products away from the electrode surface [10].

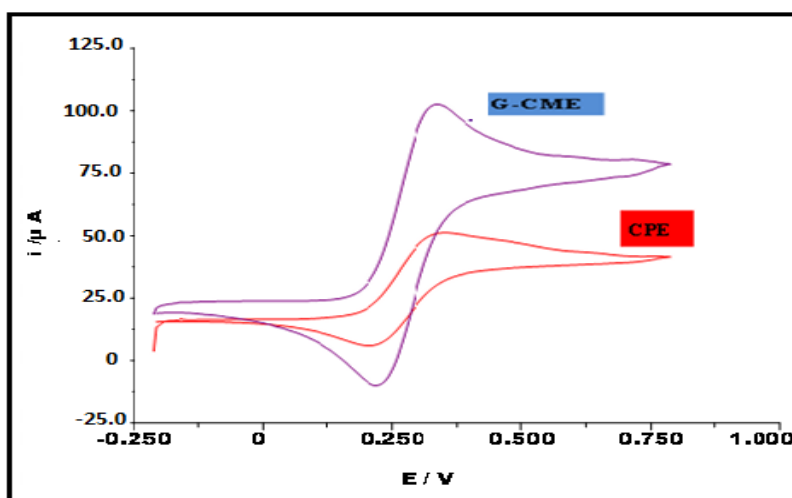


Fig. 3: Cyclic voltammetry of 6mM $\text{K}_3\text{Fe}(\text{CN})_6$, in 0.1M KNO_3 at CPE and G-CME

The surface area of the two electrodes CPE and G-CME of the same nominal bore size were found out using 6mM of mixture of $\text{K}_3\text{Fe}(\text{CN})_6$ and $\text{K}_4\text{Fe}(\text{CN})_6$ system in 0.1N KNO_3 . The surface area of both the electrodes CPE and G-CME were calculated using Randles-Sevcik [11] equation and were found to be 0.0159 cm^2 and 0.0662 cm^2 respectively

3.3. Chronocoulometry (CC)

Chronocoulometry was employed to determine the kinetics and mechanism of electro-oxidation reaction of HIS ($1 \times 10^{-6} M$) at CPE and G-CME. Double-potential step chronocoulometry after point to point background subtraction was performed for The plot of charge (Q) vs. the square root of time ($t^{1/2}$) showed a linear relationship for both the molecules. From the slope and intercept of the above plot the diffusion coefficient (D_{coeff}) and Q_{ads} of HIS were estimated respectively which is given by Anson equation [12]. The calculated parameters are tabulated in Table 2. The increase in the value of slope and charge due to adsorption (Q_{ads}) for G-CME indicate greater accumulation of HIS on its surface rather than on CPE. The surface coverage (Γ^0) for the two electrodes is calculated from equation 8, where n is the number of electrons, F is the Faraday's constant (96485 C/mol) and A is the area of the electrode surface (cm^2).

Molecule	Electrode	Slope ($10^{-4} \mu C/s^{-1/2}$)	Intercept Q_{ads} ($10^{-6} \mu C$)	Surface coverage ($10^{-9} mol/cm^2$)	Diffusion Coefficient ($10^{-6} cm^2/sec$)
HIS	CPE	2.42	3.2	2.47	1.562
	G-CME	11.27	72.8	9.51	6.05

Table 2: Chronocoulometry of $1 \times 10^{-6} M$ HIS

$$Q_{ads} = nFA \Gamma^0 \tag{1}$$

It is observed from the Table 2 that the surface coverage in the case of G-CME is greater than for CPE indicating the sensitivity of the HIS towards the modified electrode.

3.4. Determination of HIS by Differential Pulse Voltammetry (DPV):

An analytical procedure is worked out for determining HIS. The linear working range (LWR), empirical limits of detection (LOD) ($S/N=3$), linear regression equation (LRE) and correlation coefficient (r) were determined and are presented in Table 3. Fig. 3 is the voltammogram of HIS at G-CME in 0.1M Phosphate buffer (pH 4.0)

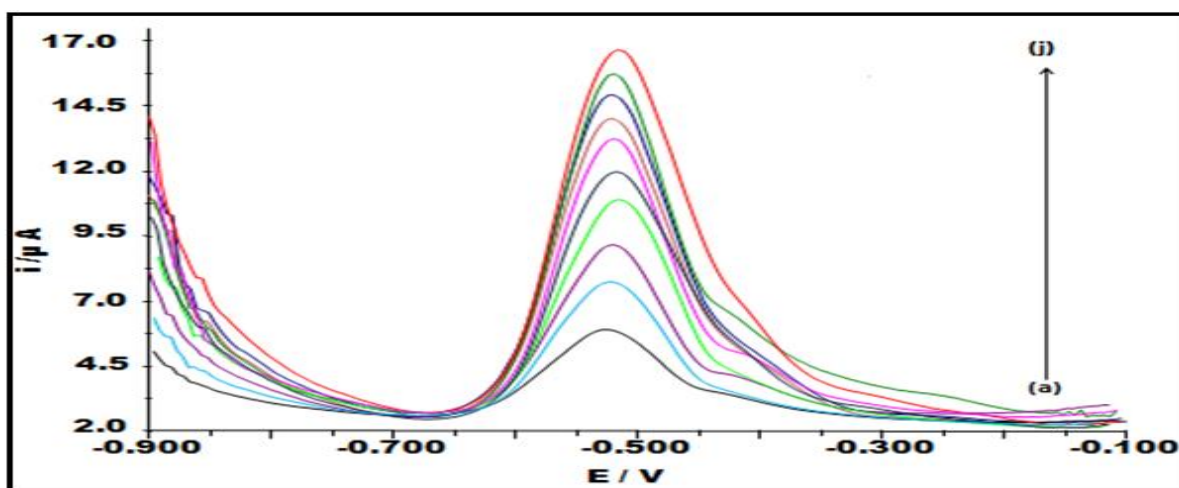


Fig.3:DPV curves obtained at G-CME for HIS at different concentrations a) 0.25 b) 0.37 c) 0.43 d) 0.52 e) 0.69 f) 0.75 g) 0.85 h) 0.9 i) 1.3 j) 1.5 μM :scan rate 10mV/s in 0.1M Phosphate buffer (pH 4.0);pulse amplitude 50mV.

Molecule	LOD	%RSD	LWR	LRE	r
Statistical data for individual molecule					
HIS	$1.26 \times 10^{-8} \text{ M}$	1.82	1.59×10^{-7} to 2.60×10^{-3}	$I_p (\mu\text{A}) = 0.028(\mu\text{M}) + 3.929$	0.989

Table 3: Analytical parameters for electrochemical determination of HIS at G-CME in 0.1M Phosphate buffer (pH 4.0)

3.8. Validation studies, interference studies and analytical applications:

For validation of the proposed method, various parameters such as repeatability, reproducibility, precision and accuracy of the analysis were obtained by performing five replicate measurements of $1 \times 10^{-6} \text{ M}$ HIS over intraday assay (single day, n = 5) and inter-day assay (for a period of 1 week). Satisfactory mean percentage recoveries (%R) and relative standard deviations (% RSD) were obtained and are presented in Table 4. The recoveries obtained confirmed high precision and accuracy of the proposed method In order to further extend the validity of the proposed method, verification of the matrix effect on HIS on determinations by DPV was studied. The influence on the peak heights of some interferences commonly present, some of them which form the major components of multivitamin pharmaceutical preparations were evaluated.

Molecule	Concentration taken (mol L ⁻¹)	Mean concentration found (mol L ⁻¹)	Mean recovery %	Bias %	Precision % RSD
HIS	Intra day				
	1×10^{-6}	$1.07 \times 10^{-6} \text{ M}$	99.4	0.54	1.67
	Inter day				
	1×10^{-6}	$1.02 \times 10^{-6} \text{ M}$	100.2	-0.37	2.21

Table 4: Precision and Bias of assay for standard HSI solution by DPV (n =5)

The tolerance limit for interfering species was considered as the maximum concentration that gave a relative error in terms of ΔI_p less than $\pm 5.0\%$ at a concentration of $1 \times 10^{-6} \text{ M}$ HIS. Five replicates of each experimental set were performed. The results showed tolerance limit of 150 fold of glucose, 50 fold for citric acid and thiamine hydrochloride, 100 fold for tartaric acid and nicotinamide, 10 fold for riboflavin and 50 fold for cyanocobalamine showing that the present modified electrode was highly selective towards the determination of HIS in the presence of common physiological interferences. The validity of the G-CME electrode was verified in the determination of HIS in various pharmaceutical preparations by standard addition method

Pharmaceutical preparation	Histidine	
	Amount of drug in the sample (mg)	Amount of drug obtained in the proposed method (mg) \pm RSD
Histidine tablet	5.0	5.0 \pm 1.3
L-Histidine capsules	600.0	598.5 \pm 2.7

Table 5: Assay of HIS in pharmaceutical preparations (n =5)

4. Conclusion

A carbon paste electrode modified with grapheme was fabricated for detection and quantification of Histidine. An acceptable linear dynamic range and detection limit were obtained. The developed differential pulse voltammetric method was applied for the determination of Histidine with good sensitivity and selectivity in pharmaceutical dosage forms. The reliability and stability of the modified electrode offers possibility to be used in quality control laboratories for identification and quantification of samples

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