L-Proline determination at Glassy Carbon Electrode

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ABSTRACT

Voltammetric determination of L-Proline (L-Pro) using Cyclic voltammetry and Differential pulse voltammetry were recorded at glassy carbon electrode within the potential window 0.0 V and 2.4 V at the physiological pH. The reference and counter electrode used were Ag/AgCl and Pt wire, respectively.The effect of supporting electrolyte and concentration of electro active species on the interaction were also studied.The results demonstrate that the catalytic oxidation of L-Pro is diffusion-controlled and irreversible Under optimized conditions, the calibration curve for L-Pro concentration was linear in $3.5 \times 10^{-8} M$ to $1.5 \times 10^{-3} M$ with a low Limit of Quantification of $9.79 \times 10^{-9} M$ and a Limit of detection of 3.23×10^{-8} M. It is shown that the prepared sensor provides a sensitive and rapid strategy for the detection of L-Proline.

Keywords: Glassy carbon electrode, L-Proline, cyclic voltammetry, Differential pulse voltammetry

INTRODUCTION

L-Proline (L-Pro) is proteinogenic amino acid which is a secondary amino acid used in the biosynthesis of protein. It is one of the twenty amino acids used in living organisms as the building blocks of proteins the Nitrogen atom is attached to the α-carbon and to chain of three carbon that together form five membered rings [1]. It is majorly found in cartilage and significance for maintaining youthful skin as well as repair muscles, connective tissue and skin damage and it is an essential component of collagen and is important for proper functioning of joints and tendons [2].

L-Pro has been quantitatively analyzed using a variety of techniques reported in the literature. These include chromatography [3,4], gas chromatography [5,6], HPLC analysis[7-9], ion exchange chromatography[10,11], spectrophotometric [12-15]. A recent study developed and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to accurately measure proline levels in human serum. It provided a foundation for further exploration of proline as a non-invasive diagnostic tool for esophageal cancer [16].L-Pro is an important molecule in both plants and humans, often used as a sign of stress or disease. In plants, when they experience stress from things like drought, salt, cold, heavy metals, or infections, proline levels tend to rise it helps researchers to understand how stressed a plant is and how well it can tolerate that stress using spectrophotometer.[17-19].

Despite their effectiveness, these methods are often costly, environmentally unfriendly, technically demanding, and may lack the desired sensitivity. Use of voltammetric methods to measure the concentration of L-Pro concentrations in real samples, such as winter wheat, highlighting the sensor's robustness and potential for agricultural or biological analysis using electrochemical techniques [20,21].Electrochemical techniques have emerged as an effective method for detecting L-Pro due to their simplicity, sensitivity, and cost-effectiveness. This study aims to determine the detection limit of L-Pro voltammetrically and to study its electrochemical properties by using Cyclic Voltammetry and differential pulse voltammetery.

MATERIAL AND METHODS

Chemicals:

All chemicals were of Analytical grade and were used as received without further purification. L-Proline Boric Acid, o-Phosphoric Acid, Glacial Acetic Acid, NaOH, Conc. HCl, Sodium Citrate, Citric Acid anhydrous, Disodium Hydrogen Phosphate, Potassium Dihydrogen Phosphate, Sodium acetate, Acetone were purchased from S.D. fine Chemicals, India. Double distilled water was used for the preparation of aqueous solutions having a specific conductivity 0.4 -0.9 µS.

Methods:

Preparation of Standard L -Proline Solution $(1 \times 10^{-3} M)$: 250ml Standard solution was prepared by dissolving 28.0 mg L-Proline in double distilled water as a stock solution.

Preparation of Buffer Solution:

1. BR Buffer (0.04M) : Prepare buffer solution by adding 4.948g boric acid, 4.56ml glacial acetic acid and 5.84ml of o-phosphoric acid in distilled water
2. Citric acid-sodium citrate buffer: Prepare 0.1M buffer solution in 1litre by adding 24.27g sodium citrate and 3.358g of citric acid anhydrous solution in distilled water
3. Phosphate Buffer (0.1M): Prepare buffer solution in 500ml by adding 14.1g disodium hydrogen phosphate and 11.45g of potassium dihydrogen phosphate.
4. Acetate Buffer (0.1M): Prepare 1litre by adding 7.72g sodium acetate and 0.352ml acetic acid.
5. Potassium Hydrogen Phthalate (0.1M) : Prepare 500ml by adding 10.2 g of KHP and adjusting to the required pH by adding KOH or HCl

Preparation of different pH solution of BR buffer from BR buffer of pH 2:

Take 100ml buffer solution and then adjust at different pH from 2 to 8 with NaOH or HCl. Dilute 10ml standard solution with adjusted pH solution.

Preparation of Different concentration solution:

Perform serial dilutions of the stock solution to prepare lower concentration solutions from 1×10^{-3}M diluted with citrate buffer solution (0.15M) for concentration study of L-Pro.

Instrumentation:

All voltammetric measurements study has been performed on PhadkeSTAT 20 potentiostat. A three electrode system employing an Ag/AgCl (3M KCl) as reference electrode, platinum electrode as counter electrode and glassy carbon as working electrode was used. The pH measurements were performed using an ELICO LI 120 pH meter.

Determination of L-proline

Cyclic Voltammetric (CV) and Differential pulse voltammetric (DPV) studies were carried out with appropriate quantity of the analyte (L-Pro) in 50mL standard volumetric flask and then making up to the mark with Citrate buffer (Cit) pH 6.0. The solution was then transferred into an electrochemical cell and the measurements were carried out at 25 ± 0.2°C. N_2 gas purging was not required as oxygen did not interfere in the measurements.

Glassy carbon electrode (GCE) was polished with the alumina gel for 5-8 minutes to clean its surface then washed with the distilled water to remove any leftover material. A solution of $K_3Fe(CN)_6$ was tested using cyclic voltammetry(CV) from -0.2V to 1.8V, at scan rate 50mV per second and run for 10 cycles and subjected to cyclic voltammetry under same parameters, the peak separation was noted to be around 56mV. If the observed peak separation was outside the acceptable range the electrode was re-polished and the entire process was repeated until the desired electrochemical response was obtained.

RESULTS AND DISCUSSION

Effect of pH:

The effect of change in pH on peak potential for L-Pro was investigated by different pulse voltammetry from pH 2 to 8 employing Britton-Robinson (BR) buffer (0.04M) by DPV**.** Standard solution of L-Pro (1×10^{-4}M) was used to find the optimum pH of the supporting electrolyte at GCE. Fig. 1 represents the graph of Ip vs Ep for various pH of BR buffer. The plot of Ep vs pH shows a negative shift of Ep values (Fig 2) with increasing pH suggest the involvement of protons for the electro-reduction of L-Pro with the involvement of proton transfer preceding the potential determining step.

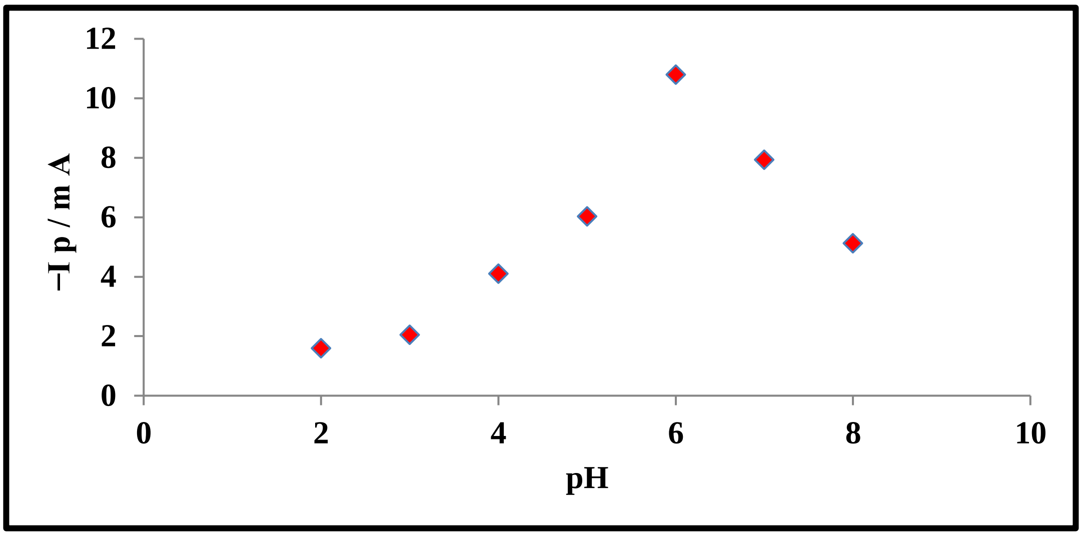

Fig. 1: Plot Ip vs pH of $1 \times 10^{-4} M$ L-Pro at; glassy carbon *vs.* Ag/AgCl; in 0.04M BR buffer ; scan rate 100mV/s at 25^0C

The peak currents were found to increase in the beginning with the increase in pH (Fig 1) showing maximum at pH 6.0 and decrease thereafter. The reduction became kinetically less favorable due to repulsive electrostatic interactions with the surface of the electrode. Therefore pH 6 was selected as the optimum pH for further studies

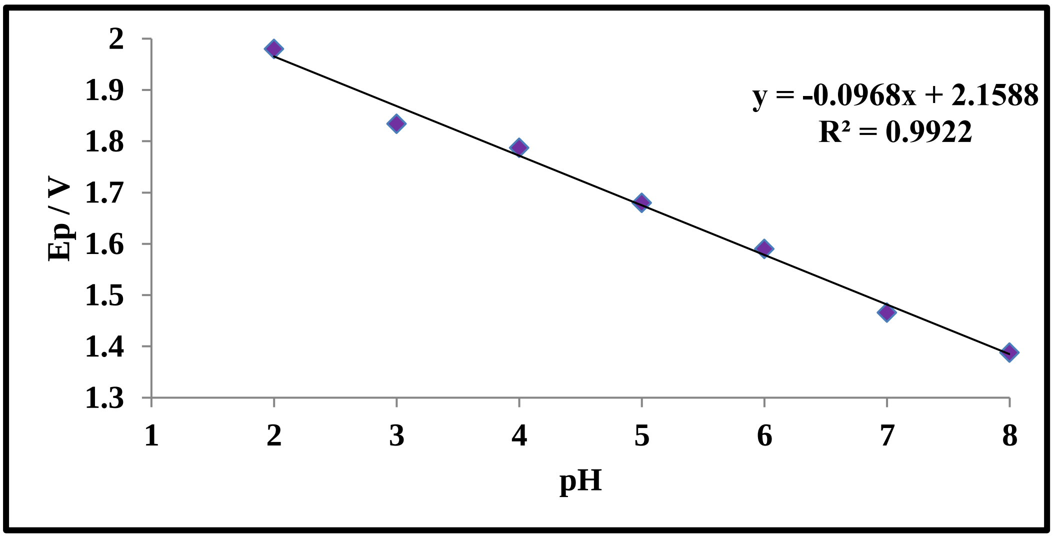

Fig. 2: Plot of Ep vs pH by Differential pulse voltammetry for reduction of $1 \times 10^{-4} M$ L-Pro at; glassy carbon *vs.* Ag/AgCl; in 0.04M BR buffer ; scan rate 100mV/s at 25^0C.

Effect of supporting electrolyte

The effects of several supporting electrolytes viz. phosphate buffer, acetate buffer, citrate buffer, BR buffer and KHP buffer at pH 6 for 1×10^{-4}M L-Pro on peak current is shown in Fig.3.

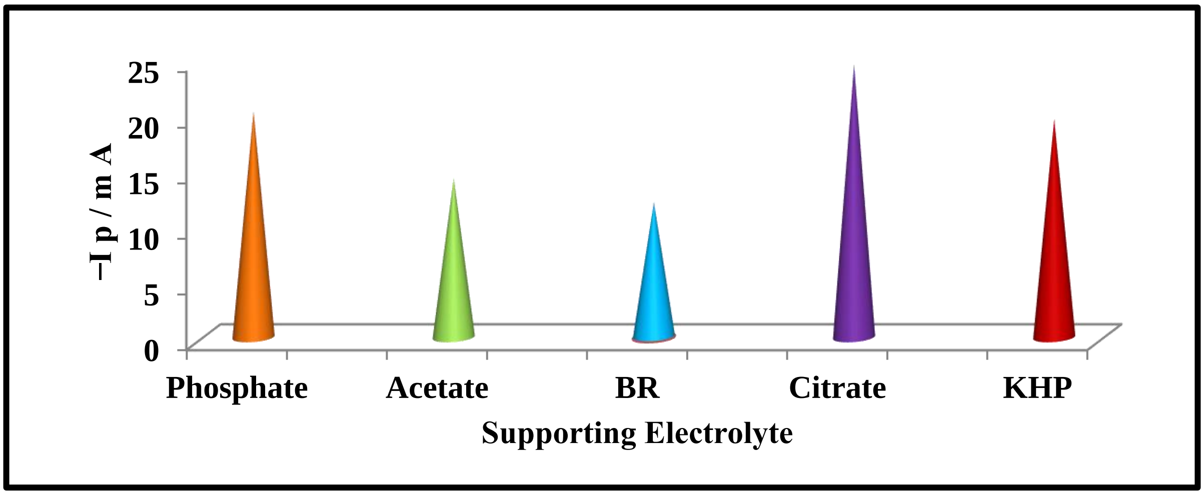

Fig. 3: Plot of Ip vs supporting electrolyte by Differential pulse voltammetry for 1×10^{-4}M L-Pro at; glassy carbon *vs.* Ag/AgCl;; scan rate 100mV/s at 25^0C.

The concentration of the buffers was taken as 0.1M except for BR buffer where the concentration was 0.04M. Amongst all the buffers used, Citrate buffer gave the best response in terms of peak current and peak shape for L-Pro**.** Thus Citrate buffer was chosen for further experiments. Further optimization of buffer concentrations was carried out by varying citric acid- sodium citrate concentration in the range from 0.05M, 0.1M, 0.15M and 0.2M the best peak response was observed for 0.15M citrate buffer (pH 6) and hence was used for the further studies.

Determination of L-Proline by Cyclic Voltammetry (CV):

Cyclic Voltammetry parameters:

Cyclic voltammetry of L-proline was observed from 1200mV to 2400mV as potential range and scan rate of 50mV/sec, scan limit is 400mV, Step size 1.0mV, initial delay of 2 sec, equilibrium time of 10sec, the current range was set to 20mA and 5 cycles were performed

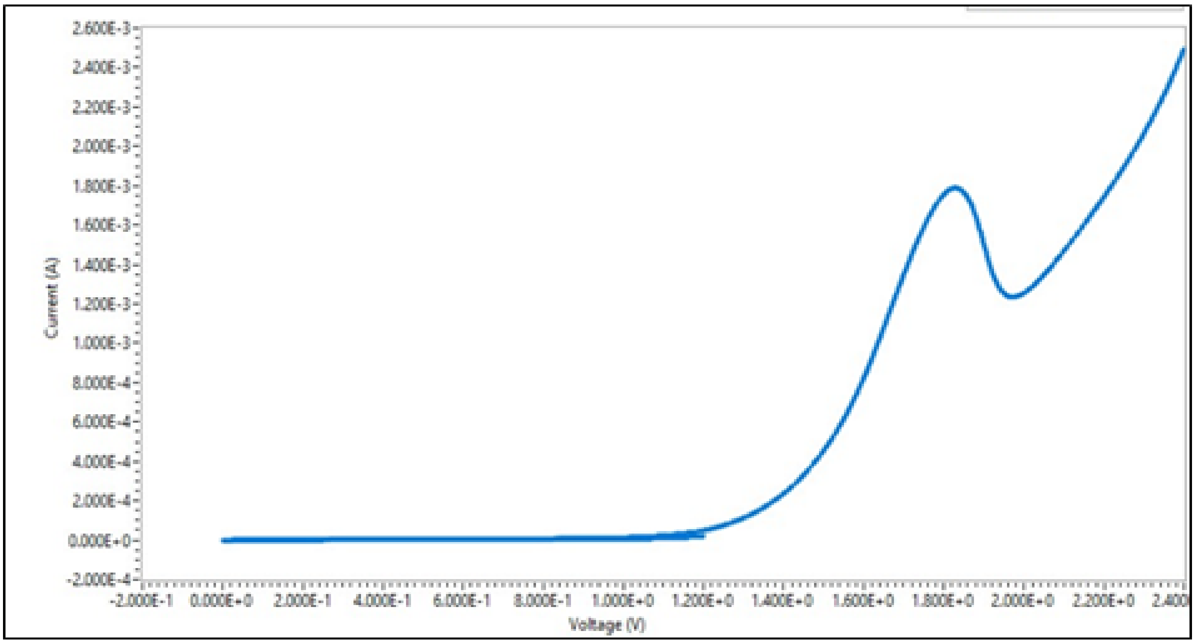

Fig. 4: CV for 1×10^{-4}M L-Pro at; glassy carbon *vs.* Ag/AgCl;; scan rate 50mV/s at 25^0C.

Effect of scan rate

The effect of scan rate between 10 and 200 mV/s for L-Pro was studied by cyclic voltammetry in 0.15M Citrate buffer (pH 6.0) and given in Fig. 5. It is observed from the figure that on increasing the scan rate, the peak current also increased A linear relationship was observed between Ip vs. v½ seen from Fig.5 indicating the electrode reaction to be diffusion controlled. The linear regression equation obtained is

$I_p = 0.021v^{½} + 4.8193$ ($R^2 = 0.9951$)

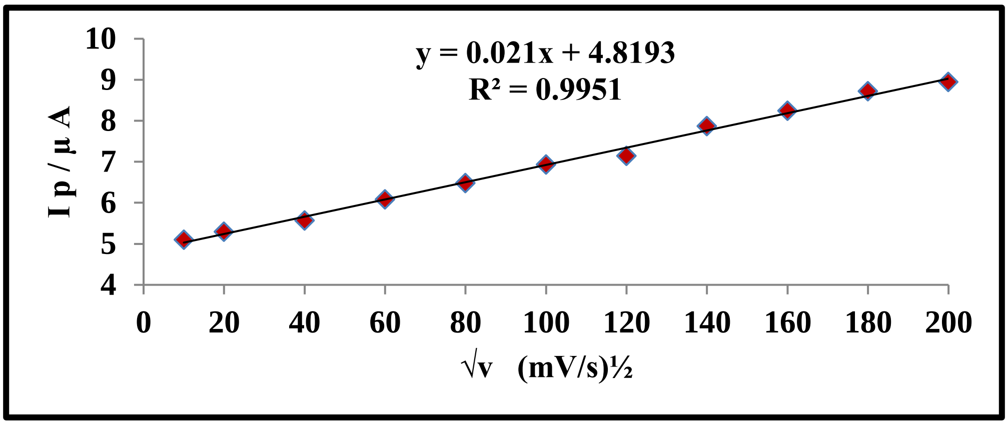

Fig 5: Plot of Ip vs √ v for 1×10^{-4}M L-Pro at; glassy carbon *vs.* Ag/AgCl;; scan rate 50mV/s at 25^0C.

Number of electrons

The number of electrons (n) involved in the oxidation reaction was calculated from the cyclic voltammetry. For a totally irreversible reaction the following equation was used

$$E_p – E_p/2 = 47.7/ n\alpha \quad \text{mV at } 25\ ^0\text{C}$$

$E_p - E_p/2$ value was found to 45 mV for L-Pro whose substitution in the above equation provided the value of αn to be 1.06,if the electron transfer coefficient (α) is assumed to be 0.5 [22]. By substituting the value of α in the above equation above the value of n is calculated to be 2.1.

Determination of L-Proline by Differential Pulse Voltammetry (DPV):

Differential pulse voltammetry parameters:

The following parameters were set for analysis of L-proline using DPV. Initial voltage was 1000mV, final voltage is 2000mV, step size is 3mV, sample period is 0.5sec, pulse size is 50mV, Pulse time is 0.1 sec. Equilibrium time is 2 sec and current range is 20mA.

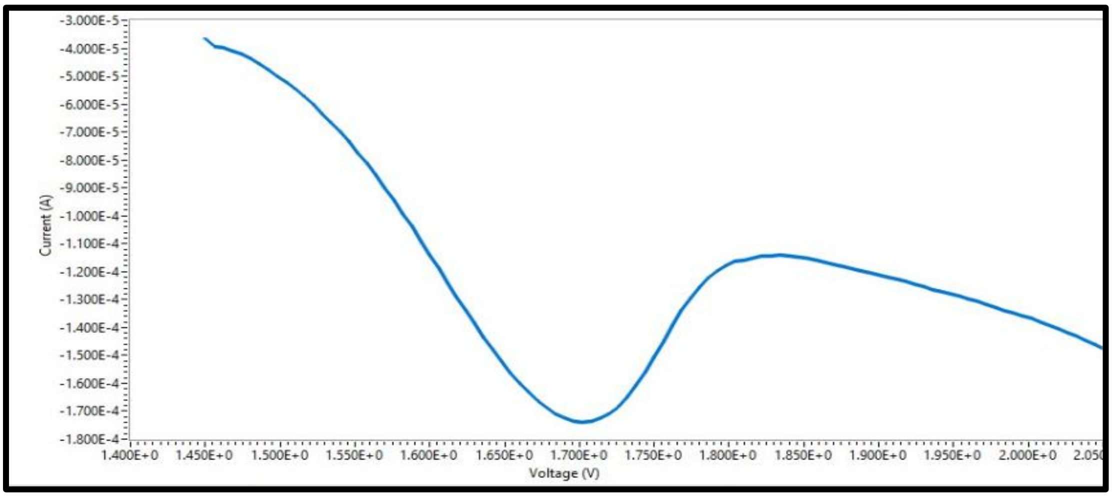

Fig. 6: DPV of 1×10^{-4}M L-Pro at; glassy carbon *vs.* Ag/AgCl;; scan rate 50mV/s at 25^0C

Effect of concentration:

The DPV technique was used for determination of L-Pro at GCE the linear working range (LWR), empirical limit of detection (LOD) , Limit of quantification (LOQ),Linear regression equation (LRE) and correlation coefficient where determined and are presented in Table 1. Fig. 7 is Plot of L-Pro at glassy carbon electrode in 0.15M citrate buffer (pH 6.0)

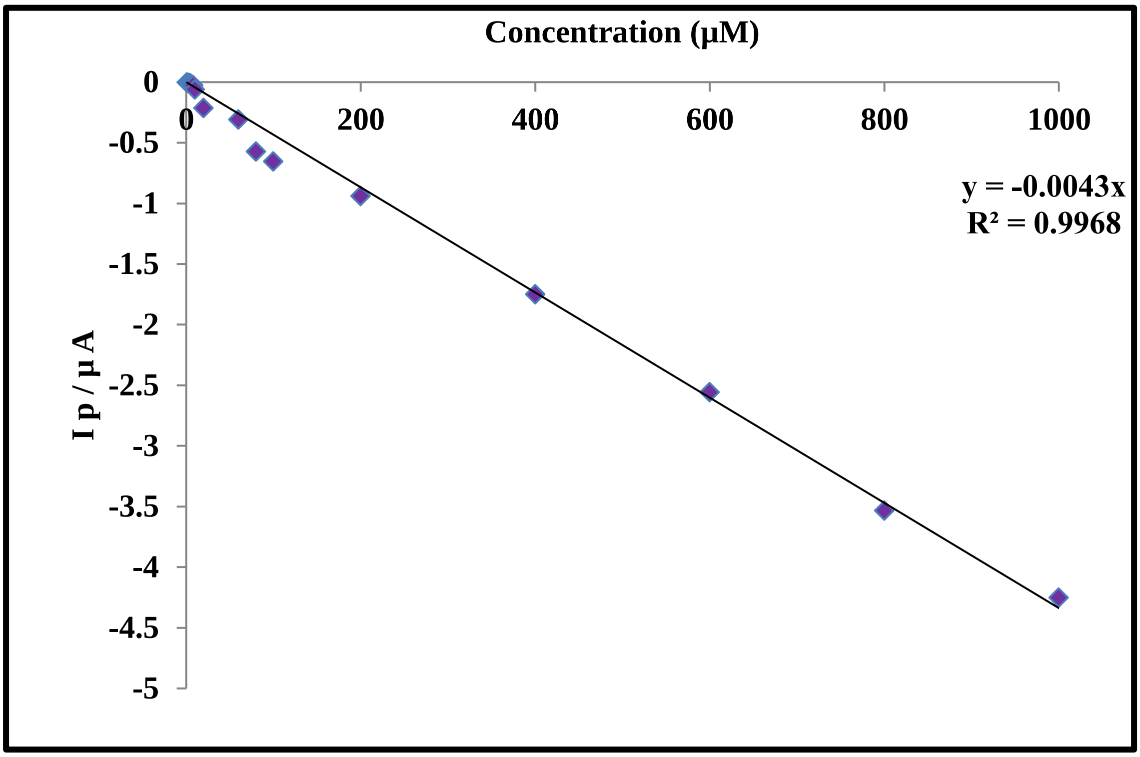

Fig.7. Plot of I_p vs Concentration (µM) of L-Pro at glassy carbon electrode in 0.15M citrate buffer (pH 6.0) at; glassy carbon *vs.* Ag/AgCl;; scan rate 50mV/s , 0.1sec, 50mV at 25^0C

LOD(M)	%RSD	LOQ (M)	LRE	LWR (M)	r
3.23×10^{-8}	± 2.21	9.79×10^{-9}	I_p (µA) = 0.0043 (µM)	$3.5 \times 10^{-8}$to 1.5×10^{-3}	0.995

Table 1: Analytical parameters for electrochemical determination of L-Pro at glassy carbon electrode in 0.15M citrate buffer (pH 6.0) at; glassy carbon *vs.* Ag/AgCl;; scan rate 50mV/s , 0.1sec, 50mV at 25^0C

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 1×10^{-4}M L-Pro over intraday assay (single day, n = 4) 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 2. The recoveries obtained confirmed high precision and accuracy of the proposed method In order to further extend the validity of the proposed method by verification of the matrix effect on L-Pro on determinations by DPV was studied.

Molecule	Concentration taken (mol L^{-1})	Mean concentration found (mol L^{-1})	Mean recovery %	Bias	Precision % RSD
L-Pro	**Intra day**				
	1×10^{-4}	$0.94 \times 10^{-4} M$	94.0	0.06	2.5
	Inter day				
	1×10^{-4}	$0.97 \times 10^{-4} M$	97.0	0.03	1.9

Table 2: Precision and Bias of assay for standard L-Pro solution by DPV (n =4)

Recovery study was done by spiking standard concentration L-Pro in urine sample. From Table3 it is seen that it indicates high reproducibility and reliability of the electrode for determination of L-Pro

	L-Proline			
	Concentration of L-Pro added 10^{-5} M	**Concentration of L-Pro found 10^{-5} M**	**Recovery %**	**Average recovery (%) ± RSD**
Urine	—	ND	—	99.9 ± 2.13
	1.75	1.73	98.7	
	4.41	4.31	99.9	
	6.21	6.27	100.9	
	7.67	7.70	100.4	

Table 4: Recovery concentrations of L-Pro in urine

The influence on the peak heights of some interferents commonly present, some of them which form the major components of multivitamin pharmaceutical preparations were evaluated. The tolerance limit for interfering species was considered as the maximum concentration that gave a relative error in terms of ΔI_p less than ± 5.0% at a concentration of 1×10^{-4}M L-Pro. Five replicates of each experimental set were performed. The results showed tolerance limit of 100 fold of ascorbic acid, 100 fold for nicotinic, 50 fold for thiamine hydrochloride, 150 fold for tartaric acid, 120 fold for riboflavin and 110 fold for cyanocobalamine showing that the present modified electrode was highly selective towards the determination of L-Pro in the presence of common physiological interferents.

CONCLUSION

A glassy carbon electrode sensor was used for the detection and quantification of L-Proline . Under optimized conditions, for L-Pro the concentration was linear in $3.5 \times 10^{-8}\,M$ to $1.5 \times 10^{-3}M$ with a low Limit of quantification of $9.79 \times 10^{-9}M$. It is shown that the prepared sensor offers rapid analysis, and is ideal for on-site or in-situ testing for the detection of L-Proline. In the food and beverage industry, monitoring to determine L-Proline levels in wines. The reliability and stability of the electrode offers possibility to be used in quality control laboratories for identification and quantification of real samples.

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