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Research Article

Electro-analytical Characterization and Method Validation of Cysteine by Cyclic Voltammetry

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ABSTRACT

This study investigates the electrochemical behavior of L-cysteine using cyclic voltammetry (CV) on a glassy carbon electrode (GCE) in a phosphate buffer solution (pH 8.0). Cysteine, a biologically significant thiol-containing amino acid, exhibits an irreversible oxidation peak corresponding to the electrooxidation of its -SH group, following a diffusion-controlled mechanism. The influence of scan rate (10–70 mV/s) and pH (4.0–9.0) on the redox process was systematically examined, revealing a proton-coupled electron transfer (PCET) mechanism. The method was validated as per ICH guidelines, demonstrating excellent linearity (10–50 x10^{-3} M, $R^2 = 0.9752$), precision (%RSD < 1.12%), accuracy (recovery 98.64–103.06%), and sensitivity (LoD = 0.192 x10^{-2} M, LoQ = 0.582 x10^{-2} M). Robustness studies confirmed method stability under slight pH variations. These findings establish CV as a rapid, sensitive, and cost-effective alternative for cysteine quantification in biochemical and nutritional analysis.

Keywords: Cysteine, Cyclic voltammetry, Electrochemical oxidation, Thiol group, Glassy carbon electrode, Method validation.

INTRODUCTION

Cysteine is a sulfur-containing amino acid that plays a crucial role in various biological processes, including antioxidant defense, protein synthesis, and detoxification of reactive oxygen species (ROS).[1] It is a precursor for glutathione, a key antioxidant molecule in cellular metabolism, and is essential for maintaining cellular redox homeostasis.[2] Sunflower seeds (*Helianthus annuus*) are known to be a rich source of amino acids, including cysteine, making their quantification important for nutritional and biochemical research.[3] Its biological importance is largely attributed to the reactive thiol (-SH) group, which undergoes oxidation to form disulfide bonds (cystine) or sulfonic species under electrochemical conditions.

The accurate determination of cysteine levels in food products, pharmaceuticals, and biological samples is essential for both nutritional analysis and quality control.[4] Various analytical techniques, such as high-performance liquid chromatography (HPLC),[5] spectrophotometry,[6] and capillary electrophoresis[7] have been used for cysteine quantification. However, these methods often require complex sample preparation, expensive reagents, and lengthy analysis times. Electro-analytical techniques, particularly cyclic voltammetry (CV), offer a rapid, cost-effective, and sensitive alternative for cysteine detection.[8]

Cyclic voltammetry is widely utilized for studying the redox behavior of biomolecules and has been successfully applied for the electrochemical determination of amino acids, including cysteine.[9] Previous studies have demonstrated that cysteine undergoes oxidation on modified glassy carbon electrodes (GCEs), producing well-defined electrochemical signals that can be used for quantification.[10]

The objective of this study is to optimize and validate a cyclic voltammetry-based method for the quantitative determination of cysteine in sunflower seed extracts. The electrochemical behavior of cysteine will be analyzed, and key parameters such as pH, scan rate, and accumulation potential will be optimized to enhance sensitivity and reproducibility. This study aims to contribute to the development of efficient electro-analytical techniques for plant-based amino acid analysis, providing an alternative to conventional chromatographic methods.

MATERIALS AND METHODS

Chemicals and Reagents

L-cysteine (≥98% purity), potassium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), and other analytical grade reagents were purchased from Sigma-Aldrich and used without further purification. All solutions were prepared using double-distilled water. A 0.1 M phosphate buffer solution (PBS) was prepared and adjusted to pH 8 using phosphoric acid or sodium hydroxide as required. The standard stock solution of L-cysteine (10 mM) was freshly prepared before each experiment and diluted appropriately to obtain working concentrations ranging from 10 to 500 µM.

Instrumentation

In this experiment, a compact Phadke STAT 20 potentiostat, operating with "EC-Prayog" software, was used for electrochemical analysis. The electrochemical cell consisted of a conventional three-electrode system with a glassy carbon electrode (GCE) as the working electrode, Ag/AgCl (3 M KCl) as the reference electrode and a platinum wire as the auxiliary electrode. All experiments were carried out at room temperature (25 ± 2°C).

Preparation of Working Electrode

Before each measurement, the glassy carbon electrode was polished with 0.05 µm alumina slurry on a polishing cloth, followed by thorough rinsing with distilled water. The electrode was then ultrasonicated in ethanol and water successively to remove any surface contaminants.

Experimental Conditions

Cyclic voltammetric measurements for the determination of L-cysteine were carried out using a three-electrode system. The supporting electrolyte used was a phosphate buffer solution (PBS) maintained at pH 8, which provided a stable medium for electrochemical measurements. The experiments were conducted at an optimized scan rate of 50 mV/s, which was found to yield well-defined and reproducible voltammetric peaks. A series of L-cysteine solutions ranging from 10 to 500 µM were prepared and analyzed to construct the calibration curve. All electrochemical experiments were performed at room temperature, maintained at approximately 25 ± 2°C.

RESULTS AND DISCUSSION

Cyclic Voltammetric Behavior of L-cysteine

The electrochemical oxidation of L-cysteine to form L-cystine proceeds through a two-electron, two-proton transfer mechanism, as represented in the structural transformation: two cysteine molecules ($HS\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH$) lose electrons and protons at the electrode surface to generate a reactive thiyl radical intermediate ($S^{\cdot}$), which subsequently dimerizes to form the stable disulfide bond (S-S) of cystine. This oxidation process can be written as the half-reaction: $2\ R\text{-}SH \rightarrow R\text{-}S\text{-}S\text{-}R + 2H^+ + 2e^-$, where R represents the amino acid backbone. The reaction is pH-dependent, with a standard redox potential (E°) of +0.22 V *versus* the calomel electrode at pH 8 (Figure 1). The mechanism involves the initial adsorption of cysteine thiol groups onto the electrode surface, followed by electron transfer and proton release to the solution. The resulting disulfide bridge formation is fundamental to many biological redox processes, including protein folding and antioxidant defense systems. This electrochemical transformation is reversible under reducing conditions, making it crucial for maintaining cellular redox homeostasis. The clean conversion with well-defined stoichiometry makes this system ideal for studying proton-coupled electron transfer (PCET) reactions in bioelectrochemistry.

Effect of pH

The electrochemical oxidation of cysteine shows strong pH dependence, with optimal results in phosphate buffer at pH 8.0. The oxidation potential shifts negatively with increasing pH, confirming

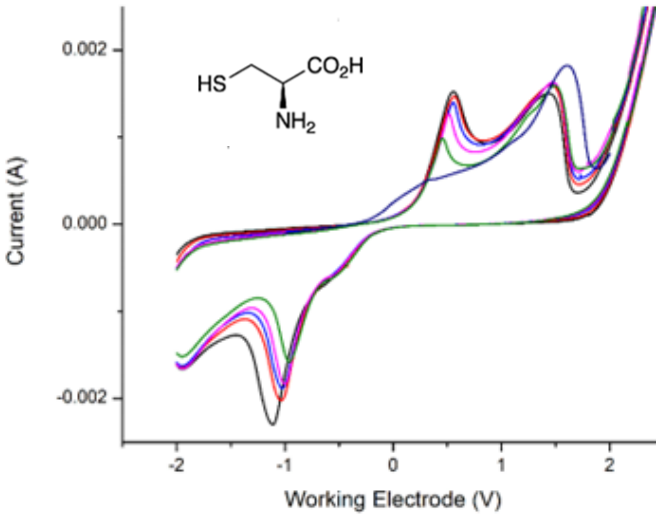

Figure 1: Cyclic voltammograms for increasing concentration of L-cysteine at phosphate buffer pH 8 and scan rate 50 V/s

a proton-coupled electron transfer (PCET) mechanism. At pH 8.0, the sharpest and most reproducible oxidation peak appears, while lower pH reduces activity due to thiol protonation and higher pH causes peak broadening (Figure 2). Phosphate buffer outperforms other systems, providing ideal conditions for cysteine detection with minimal interference, making it the preferred choice for analytical applications. These findings emphasize pH's crucial role in cysteine electrochemistry.

Effect of Scan Rate on Ep and Ip of Cysteine

The electrochemical oxidation of cysteine was studied using cyclic voltammetry over a scan rate range of 10–70 mV/s to understand the kinetics and mechanism of the reaction. The results revealed a clear

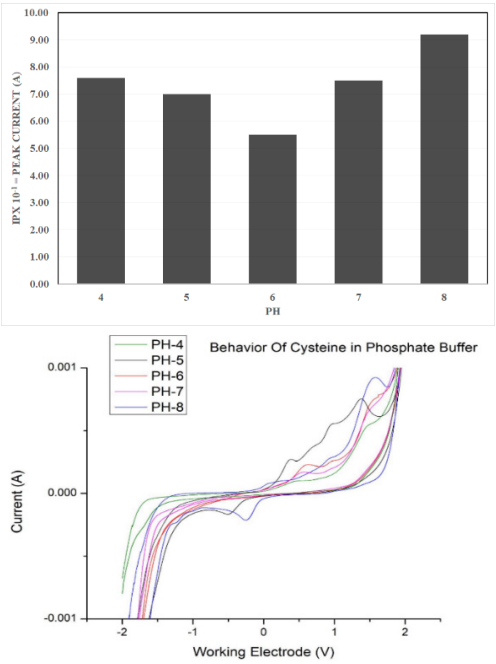

Figure 2: Cyclic voltammograms showing the effect of electrolyte and pH on cysteine oxidation. Phosphate buffer at pH 4.0 to 9.0 provides the highest peak current and stability

dependence of both oxidation peak current (Ip) and potential (Ep) on the scan rate, characteristic of a diffusion-controlled process. As the scan rate increased, Ip exhibited a linear rise from 0.0022 A at 10 mV/s to 0.0055 A at 70 mV/s, while Ep shifted positively from 0.61 V to 1.14 V, indicating quasi-reversible electron transfer behavior. The reduction peaks, though less intense and slightly shifted compared to the oxidation peaks, further supported the quasi-reversible nature of the system. To confirm the diffusion-controlled mechanism, two graphical approaches were employed. First, the cyclic voltammograms at various scan rates clearly showed an increase in peak current and positive shift in peak potential with increasing scan rate (Figure 3). Then, a plot of oxidation peak potential (Ep) *versus* the square root of the scan rate ($\sqrt{v}$) yielded a linear relationship with the equation $Ep = 9.3175\sqrt{v} - 1.5592$ and $R^2 = 0.9915$, supporting a diffusion-controlled process (Figure 4A). Additionally, a log-log plot of Ip *versus* scan rate resulted in a straight line with the equation log (Ip) = 0.5176 log (v) − 0.6065 ($R^2 = 0.9994$), with a slope close to 0.5, indicating diffusion control (Figure 4B).Furthermore, the plot of log (Ep) *versus* log (v) showed a linear trend with a slope of 0.3744 ($R^2 = 0.9941$), reinforcing the quasi-reversible nature of the electron transfer. Collectively, these findings suggest that the electrochemical oxidation of cysteine follows a PCET mechanism, governed primarily by diffusion and exhibiting quasi-reversible kinetics. The results highlight the importance of scan rate in modulating the electrochemical response and provide valuable insights into the underlying reaction mechanism, which could inform the design of sensors or catalytic systems involving cysteine.

Method Validation

The developed method of cysteine was validated as per ICH guidelines by evaluating parameters such as linearity, range, system suitability, specificity, accuracy, precision, limit of detection (LoD), limit of quantification (LoQ), robustness, and solution stability. Linearity and range confirmed the method's capability to produce proportional and reliable results. System suitability ensured proper instrument performance, while specificity verified no interference from other components. Accuracy and precision demonstrated the method's correctness and repeatability. LoD and LoQ established the method's sensitivity, and robustness confirmed reliability under slight variations. Solution stability ensured analyte integrity during analysis.

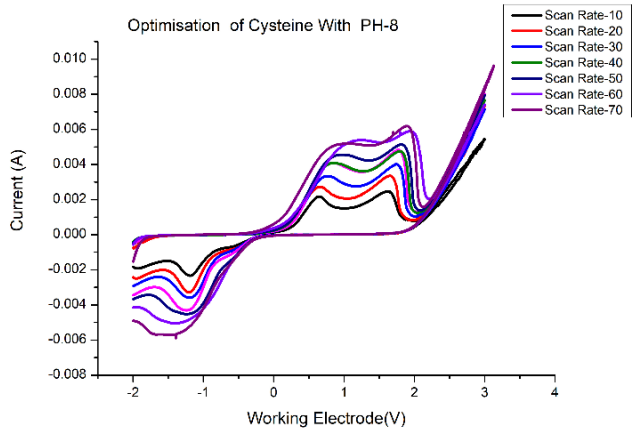

Figure 3: The cyclic voltammograms of the electrochemical oxidation of cysteine in phosphate buffer (pH 8.0) at different scan rates (10–70 mV/s)

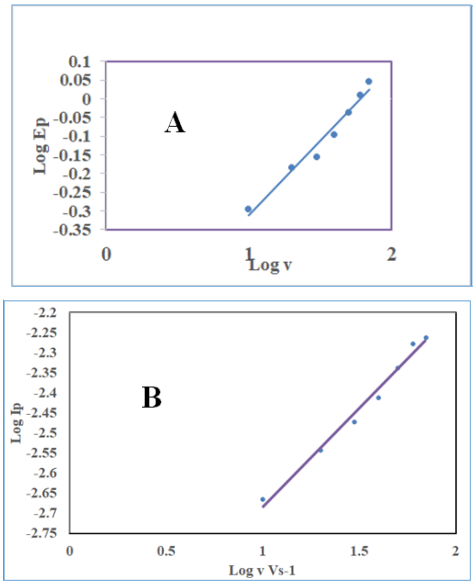

Figure 4: (A) Dependence of oxidation peak potential of cysteine on the square root of scan rate. (B) Linear relation between logarithm of peak current and logarithm of scan rate

Linearity

The linearity of the proposed cyclic voltammetric method for the determination of cysteine was evaluated in accordance with the ICH Q2(R1) guidelines. The study was carried out using a 0.1 M sodium acetate buffer as the supporting electrolyte, with a scan rate of 50 mV/s and a sampling interval of 1-mV. A series of cysteine solutions in the concentration range of 0.2 $x10^{-2}$ M to 1.2 2 $x10^{-2}$ M were analyzed, and the corresponding oxidation peak currents (first peak) were recorded. The results indicated a positive linear relationship between the concentration of cysteine and the peak current response. The calibration curve exhibited a regression equation of Ip = 49 *Ccys* + 1.024 with a correlation coefficient ($R^2 = 0.9752$), demonstrating a strong linear correlation. This suggests that the method is capable of producing results that are directly proportional to the analyte concentration within the specified range. The high degree of linearity confirms the suitability of this electro-analytical method for the quantitative determination of cysteine and supports its application in routine analysis (Figure 5).

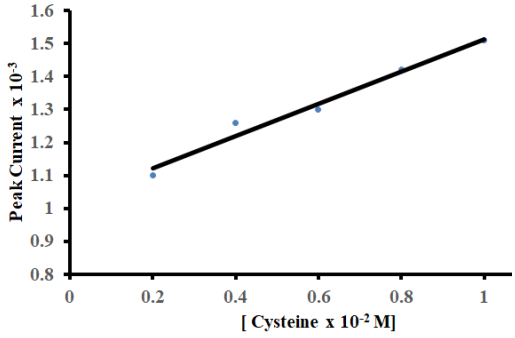

Figure 5: Calibration plot of peak current *versus* varying concentrations of L-cysteine, obtained by cyclic voltammetry at pH 8.0

Precision

The precision of the developed electrochemical method for cysteine was assessed using intra-day and inter-day measurements. Intra-day precision showed very low %RSD values (0.089–0.572%), indicating excellent repeatability within the same day. Inter-day measurements taken over three days also demonstrated good reproducibility, with %RSD values ranging from 0.089 to 1.12%. These results confirm that the method is both precise and reliable for the quantification of cysteine (Table 1).

Accuracy

The accuracy of the developed electro-analytical method for the determination of cysteine was evaluated through recovery studies at three different concentration levels, as presented in Table 2. Known amounts of cysteine standard solution (0.200, 0.600, and 1.200 × 10^{-2} M) were added, and the amount recovered was determined in triplicate (n = 3) for each level. The percentage recoveries ranged from 98.64 to 103.06%, with mean recovery values of 101.02 ± 1.02% (RSD 1.01%) for 0.200 × 10^{-2} M, 100.11 ± 1.37% (RSD 1.37%) for 0.600 × 10^{-2} M, and 101.75 ± 1.13% (RSD 1.11%) for 1.200 × 10^{-2} M. These results indicate that the method provides an accurate estimation of cysteine, with recoveries close to 100% and low relative standard deviations, demonstrating minimal experimental error. Hence, the recovery study confirms the method's high accuracy and suitability for quantitative analysis of cysteine in standard solutions (Table 2).

The LoD and LoQ

The LoD and LoQ were calculated using the standard deviation of the blank (σ) and the slope of the calibration curve (S), according to the formulas

$LoD = 3.3 \cdot \sigma / S$ $LoQ = 10 \cdot \sigma / S$

The LoD and LoQ were calculated in accordance with ICH Q2(R1) guidelines using the standard deviation of the response and the slope of the calibration curve. Based on the regression equation (Ip = 49 *Ccys*+ 1.024) and the calculated standard deviation of the residuals (σ = 0.0285), the LoD and LoQ were determined to be 0.192 x10^{-2} M and 0.582x10^{-2} M, respectively. These results indicate that the developed method is sensitive enough to detect and accurately quantify cysteine at micromolar concentrations, further supporting its suitability for routine analytical applications.

Table 1: The repeatability, intra-day and inter-day precision results

Cysteine 10^{-2}*x M*	*Intra-day Ip SD (%RSD)*	*Inter-day measurement Ip±SD (%RSD)*		
		Day 1	*Day 2*	*Day 3*
0.200	1.124 ± 0.001 (0.089%)	1.124 ± 0.001 (0.089%)	1.123 ± 0.0015 (0.136%)	1.123 ± 0.001 (0.089%)
0.600	1.323 ± 0.0075 (0.572%)	1.323 ± 0.0075 (0.572%)	1.323 ± 0.0060 (0.455%)	1.323 ± 0.0075 (0.572%)
1.200	1.621 ± 0.0087 (0.538%)	1.621 ± 0.0087 (0.538%)	1.625 ± 0.0124 (0.764%)	1.629 ± 0.018 (1.12%)

Table 2: Recovery percentage of cysteine in accuracy studies (n = 3)

No of experiments	*Amount added* 10^{-2} *x M*	*Ip (μA)*	*Amount found* 10^{-2} *x M*	*Recovery %*	*% Recovery ± SD (RDS)*
1	0.200	1.123	0.2020	101.02	101.02 ± 1.02 (1.01%)
		1.122	0.2000	100.00	
		1.124	0.2041	102.04	
2	0.600	1.322	0.6082	101.36	100.11 ± 1.37 (1.37%)
		1.319	0.6020	100.34	
		1.314	0.5918	98.64	
3	1.200	1.619	1.2143	101.19	101.75 ± 1.13 (1.11%)
		1.618	1.2122	101.020	
		1.630	1.2367	103.061	

Table 3: Robustness of the method at slight variation from the optimized pH parameters

[Cysteine] x10^{-2} *M*	*pH= 7.8*		*pH = 8*		*pH= 8.2*	
	IP	*% cysteine*	*IP*	*% cysteine*	*IP*	*% cysteine*
0.08	1.412	98.98	1.422	101.53	1.405	97.19
0.08	1.421	101.28	1.419	100.77	1.412	98.98
0.08	1.417	100.26	1.414	99.49	1.42	101.02
Mean	1.4166	100.17	1.4183	100.59	1.4123	99.063
SD	0.0045	1.1524	0.0040	1.0309	0.0075	1.9163
%RSD (%)	0.318	1.15	0.285	1.02	0.531	1.93

Robustness

The robustness of the developed cyclic voltammetric method for cysteine was assessed by introducing slight, deliberate variations in the buffer pH (± 0.2 units from the optimized pH 8.0) to evaluate the method's stability under minor experimental changes. The influence of these variations on the key electrochemical parameters peak potential (Ep) and peak current (Ip) was closely monitored. The results revealed that the changes in pH did not cause any significant deviation in either Ip or the calculated percentage of cysteine, with %RSD values for Ip ranging from 0.285 to 0.531% and for %cysteine from 1.02 to 1.93%. These low %RSD values, all within acceptable limits, confirm the precision and consistency of the method across the tested pH range. Thus, the method demonstrated excellent robustness, maintaining its reliability and accuracy despite slight fluctuations in buffer pH, which is essential for its practical applicability in routine analysis. The summarized results of robustness testing are presented in Table 3.

CONCLUSION

The present study successfully demonstrates the electro-analytical characterization and method validation of L-cysteine using cyclic voltammetry (CV) on a glassy carbon electrode in phosphate buffer solution (pH 8.0). The oxidation of cysteine, attributed to its thiol (-SH) group, follows a diffusion-controlled and PCET mechanism. Electrochemical parameters such as pH and scan rate significantly influence the redox behavior, with pH 8.0 offering optimal peak resolution and sensitivity. The oxidation peak current exhibited linear dependence on the square root of scan rate and cysteine concentration, confirming the diffusion-controlled nature of the process.

The method was rigorously validated according to ICH guidelines. It demonstrated excellent linearity, high precision (%RSD < 1.12%), satisfactory accuracy, and robust sensitivity. The method also proved stable under minor variations in analytical conditions. Overall, cyclic voltammetry offers a reliable, rapid, and cost-effective approach for cysteine quantification. This validated method holds strong potential for application in food analysis, biochemical research, and quality control of nutraceuticals and pharmaceuticals, especially those involving sulfur-containing amino acids like cysteine.

CONFLICT OF INTEREST

None declared.

SOURCE OF FUNDING

None declared.

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