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Abstract: The electrochemical oxidation of carbenicillin disodium salt (CDS) at gold electrode has been investigated for the first time using cyclic, linear sweep, and differential pulse voltammetric techniques. The dependence of the current on pH, concentration, and scan rate were investigated to optimize the experimental conditions for the determination of carbenicillin. The anodic peak was characterized and process was adsorption-controlled. The number of electrons transferred in the oxidation was calculated. A differential pulse voltammetry method with good precision and accuracy was developed for the determination of carbenicillin. The anodic peak current varied linearly with carbenicillin concentration in the range $1.0 \times 10^{-4}$ M to $5.0 \times 10^{-6}$ M with a limit of detection (LOD) of $6.859 \times 10^{-7}$ M and limit of quantification (LOQ) of $2.286 \times 10^{-6}$ M. This method can be employed in clinical analysis, quality control, and routine determination of drugs in pharmaceutical analysis.

Subjects: Analytical Chemistry; Chemistry; Physical Sciences

Keywords: carbenicillin; voltammetry; gold electrode; oxidation

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PUBLIC INTEREST STATEMENT

The electrochemical oxidation of carbenicillin disodium salt at gold electrode has been investigated using cyclic, linear sweep, and differential pulse voltammetric techniques. The dependence of the current on pH, concentration, and scan rate were investigated to optimize the experimental conditions for the determination of carbenicillin. The anodic peak was characterized and process was adsorption controlled. The number of electrons transferred in the oxidation was calculated. A differential pulse voltammetry method with good precision and accuracy was developed for the determination of carbenicillin. This method can be employed in clinical analysis, quality control, and routine determination of drugs in pharmaceutical analysis.
1. Introduction
Drug analysis is one of the important tools for drug quality control. Carbenicillin disodium salt (CDS) is a bacteriolytic antibiotic belonging to the carboxypenicillin subgroup of the penicillins. Relative to benzylpenicillin, Carbenicillin has a relatively great spectrum of activity against Gram-negative bacteria such as Pseudomonas aeruginosa, but a lower activity against Gram-positive bacteria (Reynolds, 1996). Its bactericidal mode of action is analogous to that of benzylpenicillin, which acts on bacteria by inhibiting bacterial cell wall synthesis. The carboxypenicillins are susceptible to degradation by beta-lactamase enzymes, although they are more resistant than ampicillin to degradation. Carbenicillin is also more stable at lower pH than ampicillin. The antibiotic is highly soluble in water and is acid-labile. It is a semi-synthetic analog of the naturally occurring benzyl-penicillin (Scheme 1).

Various analytical methods for the therapeutic monitoring have been reported in the literature for the determination of carbeniciilin in commercial dosage form and biological fluids such as high-performance liquid chromatography (HPLC) (Womey, 1981), LC method (Naidong, Dzerk, & Lee, 1994), electrochemical (Vedel et al., 1989), and electrophoretic method (Squella & Núñez-Vergara, 1986) The main problems encountered in using such methods are either the need for derivatization or the need for time-consuming extraction procedures.

Voltammetric methods satisfy many of the requirements for such tasks particularly owing to their inherent specificity, rapid response, high sensitivity, low cost, simplicity, and relatively short analysis time for the determination of organic molecules, including drugs and related molecules in pharmaceutical dosage forms and biological fluids (Zima, Švancara, Barek, & Vytřas, 2009).

Electrochemical methods, especially differential pulse voltammetry (DPV) make it possible to decrease the analysis time as compared to the time exhausted chromatographic methods (Erk, 2004). The advantages of DPV over other electroanalytical techniques are greater speed of analysis, lower consumption of electroactive species in relation to the other electroanalytical techniques and less problems with blocking of the electrode surface. The gold electrode has been widely used in electrochemical studies and electro analysis for various substrates for a long time because of its stability, wide potential window, and fast electron transfer rate (Chen et al., 2015; Ferapontova, 2004; Roy et al., 2015; Tak, Gupta, & Tomar, 2014; Xia & Hu, 2005).

To the best of our knowledge, till date, there is no literature on the voltammetric method for the determination of CDS. The aim of this study is to establish the suitable experimental conditions, to investigate the voltammetric behavior and oxidation of CDS at gold electrode by cyclic and linear sweep voltammetry. Further, differential pulse voltammetric (DPV) method with good precision and accuracy was developed for the determination of CDS in pharmaceutical formulations. The proposed method has advantages such has no time-consumed sample preparation step prior to drug assay, high sensitivity, rapid response, good reproducibility, and low detection limit. Hence, we here report the voltammetric behavior of CDS for the first time by cyclic, linear, and differential pulse voltammetric method at gold electrode.

Scheme 1. Chemical structure of carbenicillin disodium salt.
2. Experimental

2.1. Reagents and chemicals
Carbenicillin disodium salt was purchased from Sigma–Aldrich and used without further purification. A stock solution of CDS (10 mM) was prepared in Millipore water. The phosphate buffers from pH 3.0–11.2 were prepared according to the method of Christian and Purdy (1962). All other materials were of analytical reagent grade, and throughout the study, the solutions were prepared in Millipore water (resistivity of Millipore water is 20 MQ).

2.2. Instrumentation

- Electrochemical measurements were carried out on a CHI 630D electrochemical analyzer (CH Instruments Inc., USA).
- The voltammetric measurements were carried out in a 10 ml single compartment three-electrode glass cell with Ag/AgCl as a reference electrode, a platinum wire as counter electrode and a 2 mm diameter gold electrode as a working electrode (Part No. CHI101). All the potentials are given against the Ag/AgCl (3 M KCl).
- pH measurements were performed with Elico LI120 pH meter (Elico Ltd, India).

2.3. Area of the electrode
The area of the electrode was obtained by the cyclic voltammetric method using 1.0 mM K₃Fe(CN)₆ as a probe at different scan rates. For a reversible process, the following Randles–Sevcik formula can be used (Rezaei & Damiri, 2008)

\[
I_{pa} = 0.4463 \left( \frac{F^3}{RT} \right)^{1/2} n^{3/2} A_0 D_0^{1/2} C_0^{1/2} \nu^{1/2}
\]

where \(I_{pa}\) refers to the anodic peak current, \(n\) is the number of electrons transferred, \(A_0\) is the surface area of the electrode, \(D_0\) is diffusion coefficient, \(\nu\) is the scan rate, and \(C_0\) is the concentration, respectively, of K₃Fe(CN)₆. For 1.0 mM K₃Fe(CN)₆ in 0.1 M KCl electrolyte, \(T = 298\) K, \(R = 8.314\) J K⁻¹ mol⁻¹, \(F = 96,480\) C mol⁻¹, \(n = 1\), \(D_0 = 7.6 \times 10^{-6}\) cm² s⁻¹, then from the slope of the plot of \(I_{pa}\) vs. \(\nu^{1/2}\), relation, the electroactive area was calculated. In our experiment, the slope was \(2 \times 10^{-5}\) μA (V s⁻¹)⁰⁻⁵ and the area of electrode was calculated to be 0.2696 cm².

2.4. Analytical procedure
For reproducible results, improved sensitivity, and good resolution of voltammetric peaks, the working electrode polishing was done on micro cloths (Buehler) glued to flat mirrors. A different micro cloth was used for each size of alumina. The particle size used was 0.3, 0.1, and 0.05 μm. The final particle size was 0.05 μm. After initial cleaning of the electrode, it was only necessary to polish with 0.05 μm particle size during the time of experiments. Before transferring the electrode to the solution, it was washed with double distilled water. Cyclic voltammograms were recorded in 0.2 M H₂SO₄ at 50 mV s⁻¹ between 0 and 1.6 V, until obtaining the reproducible current-potential curves.

The parameters for differential-pulse voltammetry (DPV) were as follows: initial potential 0.8; final potential, 1.4 V; increase in potential, 0.004 V; amplitude, 0.05 V; quiet time, 2 s; sensitivity, \(1.0 \times 10^{-4}\) A/V.

3. Results and discussion

3.1. Cyclic voltammetry
In order to understand the electrochemical process occurring at the gold electrode, cyclic and linear sweep voltammetry were carried out at pH 3.0. The cyclic voltammograms obtained for 1.0 mM CDS solution at a scan rate of 50 mV s⁻¹ exhibits a well-defined irreversible anodic peak. The results are shown in Suppl. Figure 1. The cathodic peak that appeared corresponds to the reduction of gold
oxides (Barus, Gros, Comtat, Daunes-Marion, & Tarroux, 2007). It was found that the oxidation peak current of CDS showed a marked decrease during the successive cyclic voltammetric sweeps. A decrease in the oxidation peak current occurs with the number of successive sweeps. This phenomenon may be attributed to the fouling of the electrode surface due to adsorption of the oxidation product on the electrode surface. Therefore, the voltammograms corresponding to the first cycle were generally recorded.

3.2. Influence of pH
The electro-oxidation of $1.0 \times 10^{-4}$ M CDS was studied over the pH range of 3.0–11.2 in phosphate buffer solution by cyclic voltammetry which is shown in Figure 1. With the increase in pH of the solution, peak potential shifted to less positive values, and obeys the following equation:

$$E_p(V) = 1.355 - 0.054 \text{ pH; } r = 0.992.$$  

The slope is 54 mV/pH, this value is close to the theoretical value of 59 mV/pH (Martins et al., 2011) suggested that the number of electrons transferred is equal to that of the hydrogen ions taking part in the electrode reaction. From the plot of $I_p$ vs. pH, it is clear that, peak current is affected by the pH value. Because, the best result with respect to sensitivity accompanied with sharper response was obtained with pH 3.0, so pH 3.0 was selected for further experiments.

3.3. Influence of scan rate
Useful information involving electrochemical mechanism generally can be acquired from the relationship between peak current and scan rate. Therefore, the voltammetric behavior of CDS at different scan rates was also studied using cyclic voltammetry (Figure 2(A)) and linear sweep voltammetry (Figure 3). Scan rate studies were carried out to assess whether the processes on gold electrode were under diffusion or adsorption-controlled. The influence of the scan rate on the peak current showed a linear relationship in the range of 0.01 to 0.25 mV s$^{-1}$ for CV (Figure 2(B)) and in the range of 0.01 to 0.25 mV s$^{-1}$ for LSV which is of a typical adsorption controlled process (Gosser, 1993), and the equations were expressed as,

$$I_p(\mu A) = 181.1V(V s^{-1}) - 3.174, \quad r = 0.991 \text{ for CV}$$  

$$I_p(\mu A) = 166.5V(V s^{-1}) - 2.431, \quad r = 0.995 \text{ for LSV}$$

A plot of logarithm of anodic peak current vs. logarithm of scan rate gave a straight line with a slope of 0.864 for CV (Figure 2(C)) and 0.853 for LSV, which close to the theoretical value of 1.0, which is expected for an ideal reaction for the adsorption-controlled electrode process (Gosser, 1993). The equation obtained was:
The peak potential was also dependent on scan rate. The peak potential shifted to more positive values on increasing the scan rate, which confirms the irreversibility of the oxidation process and a linear relationship between peak potential and logarithm of scan rate for CV and for LSV can be expressed by the following equations:

\[ E_p = E_0^\circ + \left( \frac{2.303RT}{\alpha nF} \right) \log \left( \frac{R \alpha k_0}{nF} \right) + \left( \frac{2.303RT}{\alpha nF} \right) \log \nu \]

\( (2) \)

where \( \alpha \) (alpha) is the transfer coefficient, \( k_0 \) the standard heterogeneous rate constant of the reaction, \( n \) the number of electrons transferred, \( \nu \) (nu) the scan rate and \( E_0^\circ \) is the formal redox potential.

The values of \( \alpha \) can be easily calculated from the slope of \( E_p \) vs. \( \log \nu \). In this system, the slope is 0.064 for CV and 0.073 for LSV, taking \( T = 298 \) K, and substituting the values of \( R \) and \( F \), \( \alpha n \) was calculated. According to Bard and Faulkner (2004), \( \alpha \) can be given as,

\[ \beta \log I_p (\mu A) = 0.864 \log \nu (V \ s^{-1}) + 2.211, \quad r = 0.997 \text{ for CV} \]

\[ \log I_p (\mu A) = 0.853 \log \nu (V \ s^{-1}) + 2.153, \quad r = 0.998 \text{ for LSV} \]

The peak potential shifted to more positive values on increasing the scan rate, which confirms the irreversibility of the oxidation process and a linear relationship between peak potential and logarithm of scan rate for CV (Figure 2(D)) and for LSV can be expressed by the following equations,

\[ E_p = 0.064 + 1.267 \log \nu (V \ s^{-1}); \quad r = 0.991 \text{ for CV} \]

\[ E_p = 0.073 + 1.284 \log \nu (V \ s^{-1}); \quad r = 0.989 \text{ for LSV} \]
where \( E_{p}/2 \) is the potential where the current is at half the peak value. So, from this, we obtained the value of \( \alpha \). Further, the number of electron (\( n \)) transferred in the electrooxidation of CDS was also calculated using cyclic and linear sweep voltammetry. The value of \( k^0 \) can be determined from the intercept of the above plot if the value of \( E^0 \) is known. The value of \( E^0 \) in Equation (2) can be obtained from the intercept of \( E_p \) vs. \( \log \nu \) curve by extrapolating to the vertical axis at \( \nu = 0 \) (Yunhua, Xiaobo, & Shengshui, 2004). In our system, the intercept for \( E_p \) vs. \( \log \nu \) plot was 1.267 for CV and 1.284 for LSV methods. All the values of \( \alpha, n, E^0 \) and \( k^0 \) obtained from cyclic and linear sweep voltammetry are tabulated in Suppl. Table 1.

### 3.4. Calibration curve and detection limit

In order to develop a voltammetric method for determining the CDS, the differential pulse voltammetric method was adopted, because the peaks are sharper and better defined at lower concentrations of CDS than those obtained by cyclic voltammetry. According to the obtained results, it was possible to apply this technique to the quantitative analysis of CDS. The phosphate buffer solution of pH 3.0 was selected as the supporting electrolyte for the quantification of CDS because it gave the maximum peak current at pH 3.0. Differential-pulse voltammograms obtained with increasing amounts of CDS showed that the peak current increased linearly with increasing concentration, as
shown in Figure 4. Using the optimum conditions, a linear calibration curve was obtained for CDS in the range from $1.0 \times 10^{-4}$ to $5.0 \times 10^{-6}$ M (Inset: Figure 4). The linear equation was, $I_p (\mu A) = 0.037 C (\mu M) + 2.221; r = 0.998$.

A deviation from linearity was observed for more concentrated solutions, due to the adsorption of CDS or its oxidation product on the electrode surface. Related statistical data of the calibration curves were obtained from the nine different determinations. The sensitivity of the analytical method is determined by limit of detection (LOD) and quantification (LOQ) values. LOD is defined as the lowest concentration of the analyte that can be detected but cannot be accurately quantified. LOQ is the lowest concentration that can be precisely and accurately quantified by the proposed analytical method. The LOD and LOQ were $6.859 \times 10^{-7}$ M and $2.286 \times 10^{-6}$ M, respectively. The LOD and LOQ were calculated using the following equations (Abbar & Nandibewoor, 2012; Bagoji, Gokavi, Pattar, & Nandibewoor, 2015)

$$LOD = \frac{3s}{m} \quad LOQ = \frac{10s}{m}$$

where $s$ is the standard deviation of the peak currents of the blank (four runs), and $m$ is the slope of the calibration curve.

Precision of the method (Abbar & Nandibewoor, 2012) was investigated by intra-and inter-day determination of CDS at two different concentrations ($n = 3$) within the linear range. Accuracy of the methods expressed as bias% and RSD% for intra and inter days are as shown in Suppl. Table 2 which indicated high precision of the proposed method.

In order to ascertain the repeatability of the analysis, six measurements of $1 \times 10^{-4}$ M CDS solution were carried out using gold electrode at intervals of 30 min. The RSD value of peak current was found to be 2.160%, which indicated that electrode has good repeatability. As to the reproducibility between days, it was similar to that of within a day repeatability if the temperature was kept almost unchanged.

### 3.5. Interference studies

For the analytical application of the proposed method, the effect of interferents used in pharmaceutical preparation was examined. The effects of these interferents on the voltammetric response was carried by analyzing sample solutions containing a fixed amount of CDS ($1.0 \times 10^{-4}$ M) spiked with various excess amount of each interferent under the same experimental conditions. The experimental results (Suppl. Table 3) showed that 10-fold excess of D-glucose, sucrose, ascorbic acid, citric acid, tartaric acid, dextrose, KCl, MnSO$_4$, FeSO$_4$, and CaCl$_2$ did not interfere with the voltammetric signal of CDS.

### 4. Conclusion

The electrochemical oxidation of CDS at gold electrode in phosphate buffer solution (pH 3.0) has been investigated. The results indicated that CDS undergoes one electron and one proton transfer and the process was adsorption-controlled. The differential-pulse voltammetric procedure was used successfully to determine CDS. High percentage recovery and study of excipients showed that the method is free from the interferences of the commonly used excipients and additives in the formulations of drug. This method can be a good alternative for the analytical determination of CDS, because it is simple, sensitive, fast, accurate, and inexpensive. The proposed methods are suitable for quality control laboratories as well as pharmacokinetic studies where economy and time are essential.
Supplementary material
Supplementary material for this article can be accessed here http://dx.doi.org/10.1080/23312009.2016.1235459.

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