

Ceftazidime Dosage Investigation using Differential Pulse Polarography for Pharmaceuticals

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Abstract

An accurate direct DPP identification of ceftazidime has been made for use in drug discovery and validation. The results showed that a mercury drop of 3 mm³, an acidic medium consisting of a KH phthalate plus HCl buffer solution at pH 2, and 1 ml of NH₄Cl, 1 M as a supporting electrolyte were optimal for ceftazidime analysis.

Ceftazidime exhibited two peaks, the first at - 0.67V and the second at - 1.06V. For the determination of an unknown concentration, a standard graph of ceftazidime in concentrations ranging from 8.4×10⁻⁶ M to 2.6×10⁻⁵ M was developed. The reliability and accuracy of this technique for analysing the analyte were assessed. For ceftazidime laboratory samples, the SD was 0.1885 and the RSD% did not go above 4.573% at a concentration of 4.6 µg.ml⁻¹, 8.4×10⁻⁶ M, and at a concentration of 8.5 µg.ml⁻¹, 1.5×10⁻⁵ M, the SD was 0.35645 and the RSD% did not go over 4.345%. The peak potential E_p, and the actual number of electrons required for the reduction of ceftazidime are both calculated using the Ilkovic-Heyrovsky equation. The data show that ceftazidime requires five electrons for the reduction reaction to occur. The method has been successfully used to get ceftazidime on the market.

Keywords: Ceftazidime, DPP, Analysis.

1. INTRODUCTION

Antibiotics were previously believed to be chemical compounds produced by one type of bacterium and poisonous to other microorganisms [1]. Since their discovery and development in the 1940s, antibiotics have had a profound effect on how bacterial illnesses are handled and treated in the medical system [2]. Cephalosporins are a class of antibiotics belonging to the beta-lactam family that were first discovered in 1945 by Guiseppe Brotzu, who isolated them from the *Cephalosporium acremonium* fungus. Cephalosporins are used to treat infections caused by bacteria such methicillin-susceptible *Staphylococci*, *Haemophilus influenzae*, *Enterobacter aerogenes*, and some strains of *Neisseria gonorrhoea* [3]. Penicillin-allergic patients may benefit from cephalosporin pre- and post-operative treatment [4]. Ceftazidime is cephalosporin antibiotic of the third generation, Figure 1.

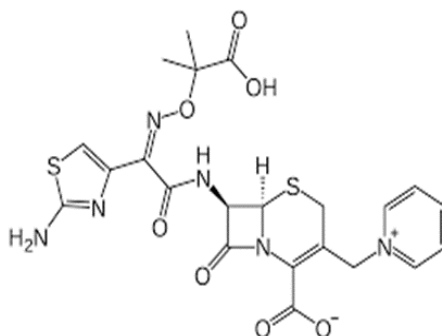


Figure 1: Structure of Ceftazidime .

Ceftazidime is significantly more effective against gram-negative bacteria. Moreover, it is more efficient against germs that may have developed resistance to earlier generations of cephalosporins [5]. Many procedures were used to quantify Ceftazidime in plasma, capsules, , urinary, and body fluid. including spectrophotometric [6][7], HPLC-UV [8] LC-MS /MS [9] as well as electrochemical techniques[10]. A significant proportion of these methods have limitations, such as high levels of complexity and the need for costly equipment.

The main aim of this research was to develop a simple but error-sensitive method for determining the purity and intensity of ceftazidime in both its purified form and in commercially available medicines.

2. EXPERIMENTAL SECTION

2.1. Apparatus

Electrochemical experiments were conducted using 99.999% pure nitrogen gas and a 797VA Computrace Metrohm, Herisau, Switzerland, polarographic analyser. DME served as the indicator electrode, while Ag/AgCl and Pt wire served as the reference and counter electrodes, respectively. A Hanna pH 211 pH meters from Romania was used to check the levels of acidity and alkalinity in the water. The temperature of all experiments was kept constant at room temperature, 25 degrees Celsius.

2.2. Materials and reagents

All experiments were conducted with reagents, chemicals, and solvents of analytical quality. Deionized water was used in the preparation of the standard and samples. All of the materials and solvents used were of analytical purity grade; AR was gained from Fluka and BDH.

Pure form Ceftazidime was purchased from the state company for pharmaceuticals and medical, SDI, in Samara, Iraq and 1g of ceftazidime is obtainable from LDP Pharma Company. A 250 $\mu\text{g.mL}^{-1}$ concentration standard

solution of Ceftazidime substances was prepared by dissolving the appropriate 12.5 mg of standard material in 50 mL of water. By adding 10 mL of the 250 $\mu\text{g}\cdot\text{mL}^{-1}$ solution to a 50 mL volumetric flask, the concentration of the working solution was reduced from 250 to 50 $\mu\text{g}\cdot\text{mL}^{-1}$, then diluting it with pure water to appropriate concentration in a series of steps. 7.45 grams of potassium chloride, 4.23 grams of lithium chloride, and 5.35 grams of ammonium chloride were dissolved in 100 millilitres of deionized water to produce 1 M solutions. To generate KH phthalate plus HCl buffer, combine 50 ml of 0.2 M KH Phthalate with 0.4 ml of 0.2 M sodium hydroxide, and then add sufficient deionized water to reach 200 ml. For the final pH adjustment, a sensitive pH meter was used [11].

2.3. General polarographic procedure

After transferring ceftazidime to a polarographic cell, 1 ml of NH_4Cl electrolyte solution, 1 ml of HCl (0.2 M) to lower the pH to 2, and 1 ml of KH Phthalate plus HCl buffer were added. The mixture was diluted with deionized water to produce standard solutions. The total volume of the cell was 20 ml after the addition of all substances. The oxygen was eliminated from the cell by degassing it with ultra-pure nitrogen for five minutes. At least two polarograms were recorded from -0.0 to -1.8 mV, and the calibration plots were used to determine the amount of Ceftazidime in the sample solutions.

2.4. Analysis

Under ideal conditions, the polarogram for ceftazidime, Figure 2 shows two peaks: the first at an applied potential of -0.67V and the second at an applied potential of -1.06V . An investigation of ceftazidime solution was carried out using a 797VA Computrace- Metrohm polarography instrument, and the optimum experimental conditions were determined. Standard calibration curves were generated using the Least Squares Method, M.L.S., and were used to determine the concentration of ceftazidime in the range of 4.6 and 14.5 $\mu\text{g}\cdot\text{mL}^{-1}$, $8.4\times 10^{-6}\text{ M}$ to $2.6\times 10^{-5}\text{ M}$ [12] and measuring ceftazidime levels, Figures 3. The regression equation analyzed unknown-concentration pharmaceutical samples. Laboratory samples showed the regression equation's reliability.

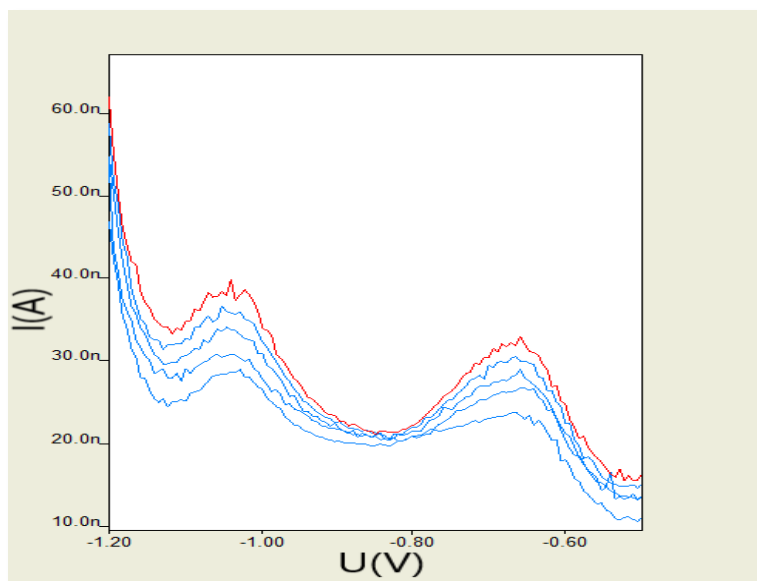
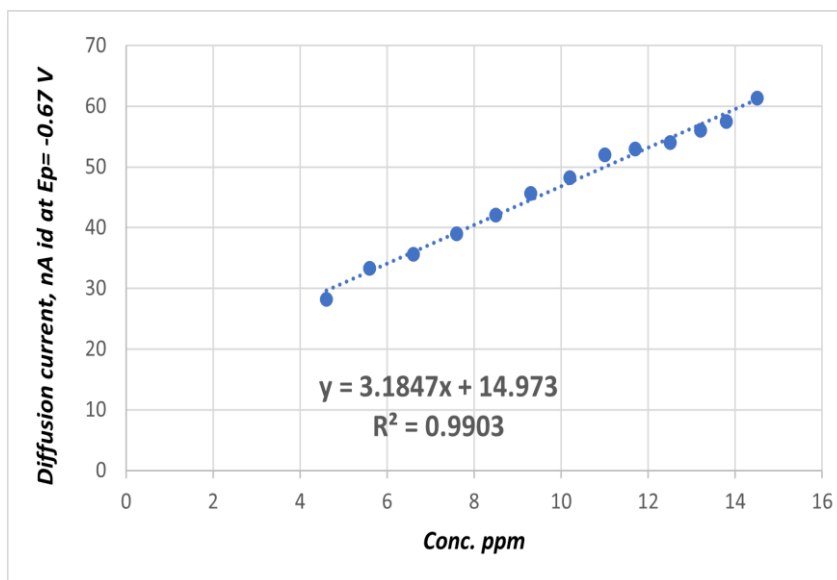


Figure 2: Polarograms of ceftazidime analysis.

Figure 3: Ceftazidime's standard titration curve between 4.6 and 14.5 $\mu\text{g.mL}^{-1}$ concentration.

3. RESULTS AND DISCUSSION

3.1 Initial CEFTAZIDIME investigation

Ceftazidime was evaluated using Direct current, DC, Differential pulse polarography, DPP and Static Mode Dropping Electrode, SMDE modes with DME which can be used to select an alternative to more expensive or time-consuming analysis methods. The polarograms showed that DPP mode with DME is accurate and precise and fast, Figure 4.

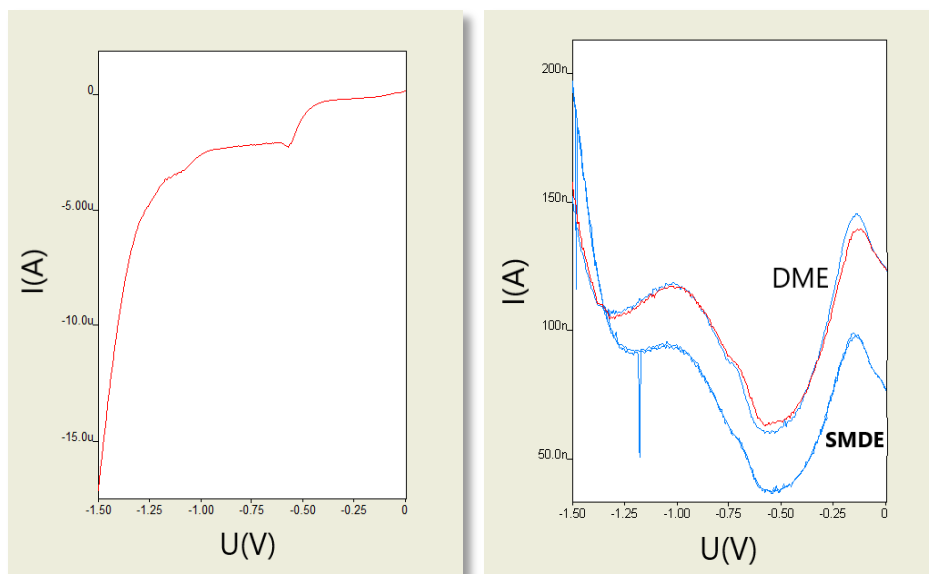


Figure 4: Ceftazidime's DC, SMDE and DPP Polarograms

3.2 Optimizing DPP

a. The effect of hydrogen number, pH

Ceftazidime samples with pH 2, 4, 7 and 9 were analysed. The acidic medium was ideal because it produced a sharp peak at a constant half-wave voltage value $E_{1/2}$, while neutral and basic media reduced peak sensitivity and shifted the peak toward more negative voltages, Figure 5.

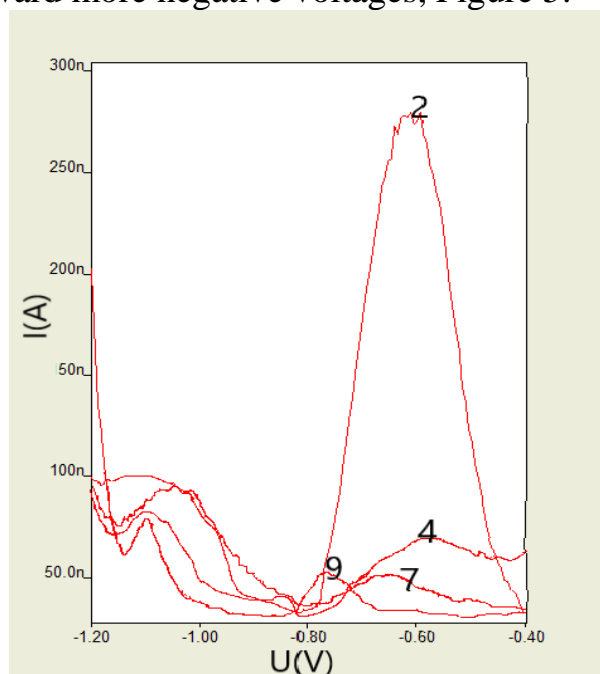


Figure 5: Polarograms of ceftazidime solutions with different pH levels.

b. Effect of Buffers

Citrate phosphate, hydrochloric acid-potassium chloride, and potassium phthalate-HCl buffers were chosen. to assess ceftazidime qualitative and quantitative analysis. Ceftazidime's polarographic response was sharp and well-defined at -0.67V applied potential in the optimal buffer solution of KH Phthalate and HCl at $\text{pH}=2$, Figure 6 .

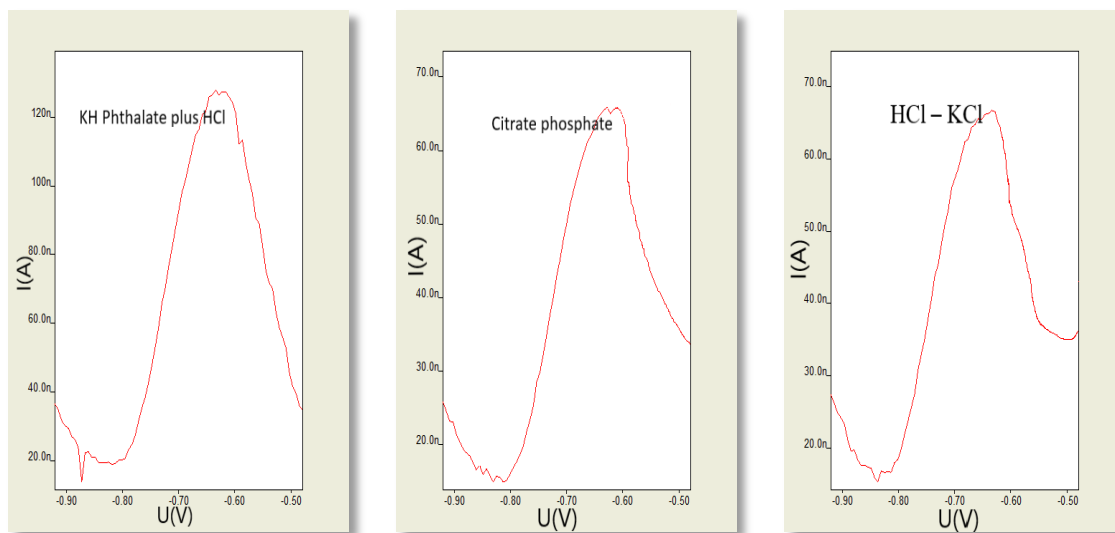


Figure 6: Ceftazidime DPP peaks at different Buffers.

c. The impact of the supporting electrolyte

Adding the supporting electrolyte to the polarograph cell solution at a high enough concentration controls migration current and optimizes electrical signal [13]. This study used three electrolytes. Lithium, ammonium, and potassium chlorides the results showed that ammonium chloride produced the best diffusion current signal, and the diffusion current value increased as the supporting electrolyte concentration decreased, as did the peak voltage stability as the type and concentration changed, Figure 7.

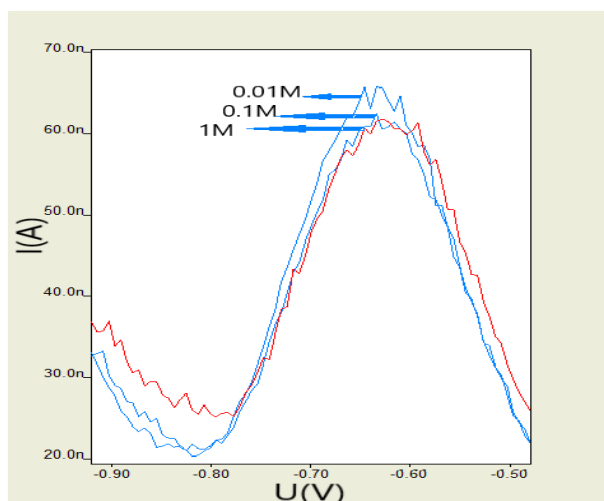


Figure 7: Polarograms of ceftazidime drug analysis with varied NH_4Cl concentrations.

3.3 Accurateness, specificity, and dignity in analysis, plus main working condition

According to the findings, the following are the optimal settings for Ceftazidime analysis, Table 1.

Table 1: Optimal settings for testing ceftazidime.

Experimental condition	Range	Appropriate conditions
Buffer	Citrate phosphate, HCl – KCl, Potassium Phthalate–HCl	Potassium Phthalate–HCl
pH	2,4,7,9	2
Supporting Electrolyte	LiCl, KCl, NH_4Cl	NH_4Cl
Solvent	Water-Methanol-Acetonitrile	D.W
Mercury drop size	1, 2, 3, 4	4
Temperature, °C	25 °C	25 °C

The reliability and specificity of this technique have been quite well through extensive testing for $4.6 \mu\text{g}.\text{ml}^{-1}$ (8.4×10^{-6} M) and $8.5 \mu\text{g}.\text{ml}^{-1}$ (1.5×10^{-5} M) laboratory ceftazidime samples, The quantity is determined to be 4.122 ± 0.4679 and 8.204 ± 0.8849 , with the relative error ranging from 0.00 to 4.58 and 1.91 to 4.97, and the results confirmed appropriate values for standard error of the mean and confidence limit of the mean., Tables 2 & 3.

Table 2: The analytical results of standard sample (8.4×10^{-6} M) of ceftazidime

Initial Conc. $\mu\text{g}.\text{ml}^{-1}$	Computed Current, nA	Determined conc. $\mu\text{g}.\text{ml}^{-1}$	Absolute error	Relative error, %	SD	SEM	%RSD	C.L of the mean
4.6 $\mu\text{g}.\text{ml}^{-1}$	27.5	3.9335	-0.188	4.56	0.1885	0.10883	4.573	4.122 ± 0.4679
	28.1	4.122	0.00	0				
	28.7	4.31	0.189	4.58				
		av. = 4.122						

Table 3: The analytical results of standard sample 1.5×10^{-5} M of ceftazidime.

Initial Conc. $\mu\text{g. ml}^{-1}$	Computed Current, nA	Determined conc. $\mu\text{g. ml}^{-1}$	Absolute error	Relative error, %	SD	SEM	%RSD	C.L of the mean
8.5 $\mu\text{g. ml}^{-1}$	40.3	7.953	-0.251	3.059	0.35645	0.20580	4.345	8.204± 0.8849
	40.6	8.047	-0.157	1.91				
	42.4	8.612	0.408	4.97				
		av. = 8.204						

Application for the determination of ceftazidime in local pharmaceuticals using DPP on DME in an acidic medium, with a KH- Phthalate+HCl buffer solution at pH 2 and 1 ml of NH_4Cl (1 M) as a supporting electrolyte; The final result shows that relative error of no more than 2.42 % and an absolute error of between -0.2833 and +0.1897. This method was also used to determine the amount of ceftazidime in commercial pharmaceuticals; Table 4 shows that a 1 g vial of ceftazidime from LDP Pharma Company contains between 958.5 and 998 mg, which is the same as what was initially installed in the product.

Table 4: commercial ceftazidime.drug analysis.

Ceftazidime vial (for injection), 1g										
Initial Conc. $\mu\text{g. ml}^{-1}$	Computed Current, nA	Determined conc. $\mu\text{g. ml}^{-1}$	%Rec	Found in drug	Absolute error	Relative error, %	SD	SEM	%RSD	C.L of the mean
11.9	51.3	11.407	95.8	958.5	- 0.2833	2.42	0.20414	0.10207	1.746	11.69 ± 0.3246
	52.5	11.784	99	990	0.0937	0.8				
	52.8	11.88	99.8	998	0.1897	1.623				
	52.2	11.69	98	982	- 0.0003	0.002				
		av. = 11.6903	98.15	av. =982						

3.4 Limits of detection and quantification.

Ceftazidime's limit of detection, LOD and limit of quantification, LOQ were calculated to be 0.9948 and 3.316 $\mu\text{g. ml}^{-1}$, respectively, at signal-to-noise ratios (S/N) of 3.3 and 10, respectively [12].

3.5 The Number of shared electrons and the value of $E_{1/2}$

The Heyrovsky-Ilkovic equation, which describes cathodic reduction at 25 °C, was used to estimate $E_{1/2}$ and electron transfer in reversible or irreversible electrode processes [14].

$$E_{\text{applied}} = E_{1/2} - (0.0591/n) \text{Log} (i/i_d - i)$$

Diffusion current and applied voltage are related by $\text{Log} (i/i_d - i)$ versus applied voltage in reversible or irreversible processes, The electron count (n) appears complete in reversible processes but incomplete in irreversible processes [15].

The number of reduction electrons, n required to reduce ceftazidime was calculated. Ceftazidime's peaks at -0.67 V are indicative of its superior stability in linear growth of i_d . Our calculations show that 5 electrons are needed for Ceftazidime to undergo reduction and produce a straight line, Figure 8. DPP experiments show that ceftazidime's azomethine functional group is reduced in acidic environments. According to the data, four electrons are required for the reduction process [16] As well as the last electron used in carbonium ion reduction, Figures 9 [17].

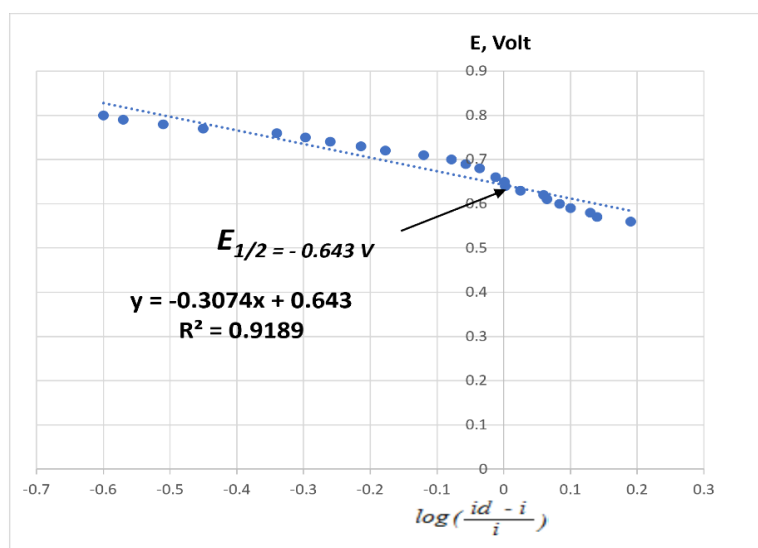


Figure 8: The Heyrovsky-Ilkovic equation for ceftazidime yields an E with a $\log i/i_d - I$ difference.

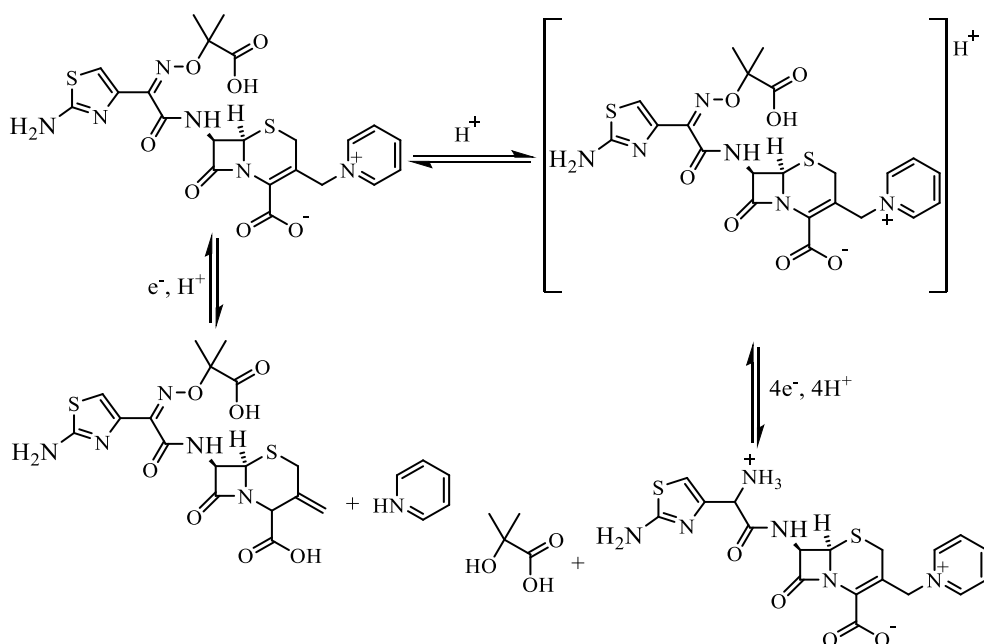


Figure 9: The proposed reduction mechanism for ceftazidime .

4. CONCLUSION

After optimizing the method's parameters, a perfect linear correlation, R was discovered between peak current and medication concentrations. The proposed method was evaluated with a commercial ceftazidime combination, and the results were as anticipated. The proposed method replaces the time-consuming and labour-intensive sample pre-treatment with a direct, simple, sensitive, and rapid analysis.

REFERENCES

- [1] Russell AD, Types of antibiotics and synthetic antimicrobial agents. 2004.
- [2] C. for D. E. and Research, "Battle of the Bugs: Fighting Antibiotic Resistance." 2016, [Online]. Available: <https://www.fda.gov/drugs/information-consumers-and-patients-drugs/battle-bugs-fighting-antibiotic-resistance>.
- [3] S. Pegler and B. Healy, "In patients allergic to penicillin, consider second and third generation cephalosporins for life threatening infections," *Bmj*, vol. 335, no. 7627, p. 991, 2007, [Online]. Available: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17991982.
- [4] N. Rao and D. Singh Rajput, "Cephalosporin Antibiotics History and Update," *IJESIR) Int. J. Sci. Innov. Res.*, 2021, [Online]. Available: <https://www.ijesir.org>.
- [5] Wikipedia contributors, "Cephalosporin." 2022, [Online]. Available: <https://en.wikipedia.org/wiki/Cephalosporin>.
- [6] M. M. M. Al-Rufaie and Z. Y. Motaweq, "Estimation of Cephalosporins

- (Ceftriaxone, Ceftazidime) Antibiotics As Pure and Pharmaceutic Forms By Color Produced Reaction in Uv-Vis Spectrophotometric Technique,” *J. Islam. Pharm.*, vol. 3, no. 2, p. 1, 2018, doi: 10.18860/jip.v3i2.6098.
- [7] A. K. Chavala, G. Bannimath, N. S. Sama, and K. V. R. Prasanth, “Spectrophotometric determination some cephalosporins containing amino group using 1, 2-napthaquinone-4-sulfonic acid sodium in pharmaceutical dosage form,” *Int. J. Pharm. Technol.*, vol. 3, no. 1, pp. 1750–1767, 2011.
- [8] P. B. and H. M. FRANCOIS JEHL*, “Hospital routine analysis of penicillins, 3rd-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid-chromatography.,” *Elsevier Sci.*, 1987.
- [9] P. Colin, L. De Bock, H. T’Jollyn, K. Boussey, and J. Van Bocxlaer, “Development and validation of a fast and uniform approach to quantify β -lactam antibiotics in human plasma by solid phase extraction-liquid chromatography-electrospray-tandem mass spectrometry,” *Talanta*, vol. 103, pp. 285–293, 2013, doi: 10.1016/j.talanta.2012.10.046.
- [10] B. Feier, A. Gui, C. Cristea, and R. Săndulescu, “Electrochemical determination of cephalosporins using a bare boron-doped diamond electrode,” *Anal. Chim. Acta*, vol. 976, pp. 25–34, Jul. 2017, doi: 10.1016/j.aca.2017.04.050.
- [11] J. N. Harman, “PH and PH measuring systems,” in *Process instruments and controls handbook*, New York: McGraw-Hill, 1985, pp. 6–126.
- [12] J. N. Miller and J. C. Miller, “Statistics and Chemometrics for Analytical Chemistry, 6th Edition,” 2010.
- [13] N. Gupta and H. Linschitz, *Hydrogen-bonding and protonation effects in electrochemistry of quinones in aprotic solvents*, vol. 119, no. 27. 1997.
- [14] Salam A. H. Al-Ameri and Bayan H. A. Al-Ameri, “Application of DPP for the determination of cefdinir in pharmaceuticals,” *Glob. J. Sci. Front. Res. B Chem.*, vol. 17, no. 1, pp. 27–32, 2017.
- [15] E. Al Rufaie, M. Abdel, and K. Hussain, “Study of Interaction between Vitamin C and Nickel Ion using a Polarographic Methods,” *Iraqi J. Sci.*, vol. 55, no. 3, pp. 878–885, 2014.
- [16] G. V. S. Reddy and S. J. Reddy, “Estimation of cephalosporin antibiotics by differential pulse polarography,” *Talanta*, vol. 44, no. 4, pp. 627–631, 1997, doi: 10.1016/S0039-9140(96)02081-4.
- [17] F. J. Jiménez Palacios, M. Callejón Mochón, J. C. Jiménez Sánchez, and J. Herrera Carranza, “Electrochemical reduction of cefepime at the mercury electrode,” *Electroanalysis*, vol. 12, no. 4, pp. 296–300, 2000, doi: 10.1002/(SICI)1521-4109(20000301)12:4<296::AID-ELAN296>3.0.CO;2-O.