Differential Pulse Polarographic Analysis of Ceftriaxone and Cefotaxime and Its Estimation in Pharmaceuticals

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Abstract
Direct DPP identification of ceftriaxone and cefotaxime in pharmaceuticals has been made for the development and validation. Based on the data results, it was determined that a 4 mm3 mercury drop size at 25°C in an acidic medium with a citrate-phosphate buffer solution at pH of 2-4 and 1 ml of LiCl, 1 M and 0.5 ml of KCl, 1 M as a supporting electrolyte yielded the most accurate findings for analysing ceftriaxone and cefotaxime Ceftriaxone had two peaks, the primary one at - 0.67V and the second at -0.95V, also cefotaxime has two peaks at - 0.67V and at -1.11V. A standard graph of ceftriaxone also cefotaxime in the concentration ranging between 3–20 µg.mL-1 was created and applied for the estimation of unknown concentration. The precision as well as accuracy of this method for determining both analytes were evaluated. In laboratory samples of ceftriaxone, the SD was 0.0866 and the RSD% did not surpass 0.805%, whereas in cefotaxime samples, the SD was 0.154 while the RSD% did not go over 2.376%. Calculations made with the Ilkovic-Heyrovsky equation reveal the true values of the peak potential, Ep also the real number of electrons needed for the reduction of ceftriaxone and cefotaxime. The results illustrate that both ceftriaxone and cefotaxime need 5 electrons for reduction reaction. This technique has been successfully implemented in commercial ceftriaxone and cefotaxime medicines.
Keywords: Ceftriaxone, Cefotaxime, DPP, Analysis.

1. Introduction

Antibiotics were assumed to be chemical compounds produced by one type of bacterium that are toxic to other microorganisms [1]. Antibiotics were discovered and developed in the 1940s and their subsequent incorporation into health care system has changed the management and fight bacterial infections [2]. In 1945, Guiseppe Brotzu isolated cephalosporins, members of the beta-lactam family of antibiotics, from the fungus Cephalosporium acremonium, which led to their original discovery. Diseases brought on by bacteria like methicillin-susceptible Staphylococci, Haemophilus influenzae, Enterobacter aerogenes and certain Neisseria gonorrhoeae are treated with cephalosporins [3]. Patients with a penicillin allergy may benefit from pre- and post-operative therapy with cephalosporins [4]. Ceftriaxone and cefotaxime are cephalosporin antibiotics of the third generation, Figure 1.

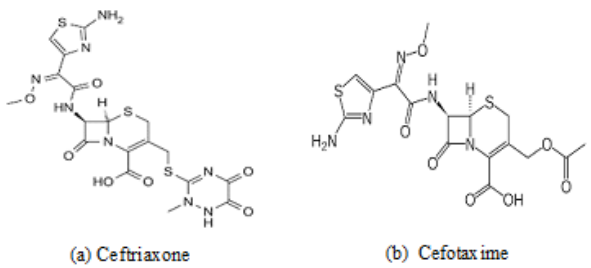

Figure 1: Structure of Ceftriaxone and Cefotaxime

They have a harsher level of efficacy against gram-negative bacteria. Furthermore, they are more effective against bacteria that may have developed resistance to previous cephalosporin generations [5]. Various methods for determining Ceftriaxone and Cefotaxime in tablets, serum, urine, bile and saliva were used, including spectrophotometric [6,7], TLC chromatography [8], LC-MS [9] as well as electrochemical techniques [10]. The majority of these published approaches have drawbacks, such as a complicated procedure, expensive instrument requirements and low detection sensitivity.

The objectives of this investigation were to create an easy and susceptible method for the qualitative also quantitative levels determining of Ceftriaxone and cefotaxime in their pure forms and trade medications.

2. 2. Experimental Section

2.1. Apparatus

A 797VA Computrace Metrohm, Herisau, Switzerland, polarographic analyser was employing for electrochemical experiments using 99.999% pure nitrogen gas. It was utilized in DME as an indicator electrode, Ag/AgCl as a reference electrode and Pt wire as a counter electrode. For pH measurements, a pH 211 pH meter, Hanna model, Romania was utilized. All experiments were conducted at a room temperature of 25 °C.

2.2. Materials and reagents

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Analytical grade reagents, chemicals, and solvents were used to complete all experiments. All the materials, solvents used were of analytical purity grade, AR achieved from Fluka and BDH; the standard and samples were prepared using deionized water.

Pure form: Ceftriaxone and Cefotaxime were purchased from the state company for pharmaceutical and medical equipment in Samara, Iraq (SDI). Ceftriaxone and cefotaxime 1 g are commercially available from LDP Pharma Company. By dissolving the correct 12.5 mg of standard material in 50 mL of water, a 250 $\mu g.ml^{-1}$ concentration standard solution of Ceftriaxone and cefotaxime substances were created. Working solutions were diluted from 250 to 50 $\mu g.mL^{-1}$ by transferring10 mL of the 250 $\mu g.mL^{-1}$ solution to a 50 mL volumetric flask. then getting through a series of dilutions with distilled water. Dissolving 7.45 g, 4.23 g, and 5.35 g of potassium, lithium, and ammonium chloride in 100 ml of deionized water made 1 M solutions. In order to make citrate buffer, 4.2 g of citric acid, 0.1 M, and 0.29 g of sodium citrate dehydrate, 0.1 M were dissolved in D.W and adjusted to 100 ml final volume. On the other hand, to obtain a pH ≈ 2 of Citrate-Phosphate buffer, combine 44.6 mL of 0.1 M citric acid with 5.4 ml of 0.2M dibasic sodium phosphate and dilute to 100 ml with D.W [11].

2.3. General polarographic procedure

Following the transfer of an amount of ceftriaxone and cefotaxime to a polarographic cell, the mixture was then diluted with deionized water. For Ceftriaxone and Cefotaxime, one millilitre of Citrate-Phosphate buffer with a pH range of 2-4 was added, and as supporting electrolytes, one millilitre of LiCl and 0.5 millilitre of KCl were respectively added for Ceftriaxone and Cefotaxime. After all of the other additions, the total volume of the cell came out to be 20 ml. The oxygen was eliminated from the cell by first degassing it for five minutes with high-purity nitrogen. At least two recordings of the polarograms were made from –0.0 to –1.8 mV, and the amount of Ceftriaxone and Cefotaxime in the sample solutions was resolved using the calibration plots.

2.4. Analysis

Ceftriaxone's polarogram, Figure 2 reveal two peaks, first one at the applied potential of - 0.67V and the second at -0.95V, on the other hand, cefotaxime showed two peaks, although one was at - 0.67V and the other was -1.11V under optimal conditions, Figure 3. The optimal experimental conditions were determined using a 797VA Computrace- Metrohm polarography device to examine ceftriaxone and cefotaxime solutions. Ceftriaxone and cefotaxime concentrations were calculated using standard calibration curves in the range of 3 to 20 µg. ml-1 that were generated using the Least Squares Method, M.L.S [12], Figures 4 & 5, as well as being utilized in the process of quantifying the levels of ceftriaxone and cefotaxime. Samples of pharmaceuticals with an unknown concentration were analyzed via the regression equation. The reliability of the regression equation was observed by analyzing samples created in a laboratory.

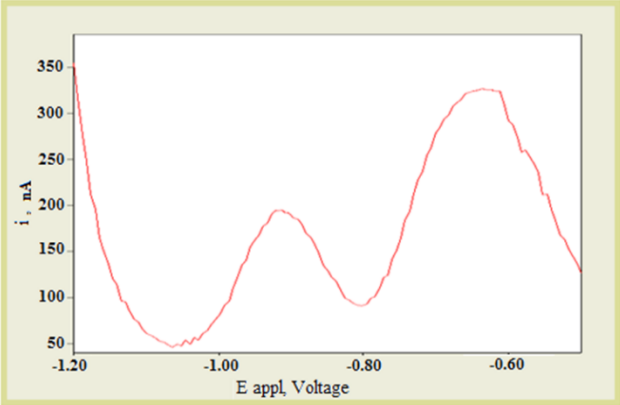

Figure 2: Polarogram of ceftriaxone at 250 µg.ml-1 concentrations.

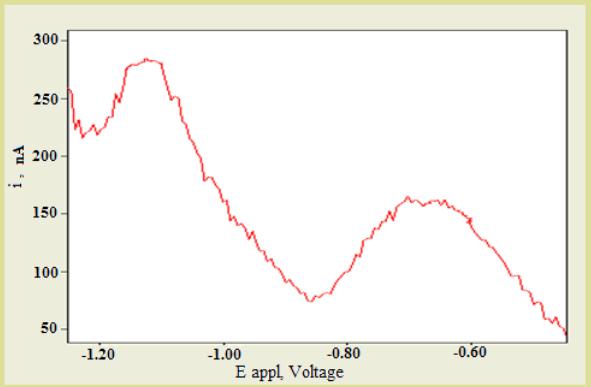

Figure 3: Polarogram of cefotaxime at 250 µg.ml-1concentrations.

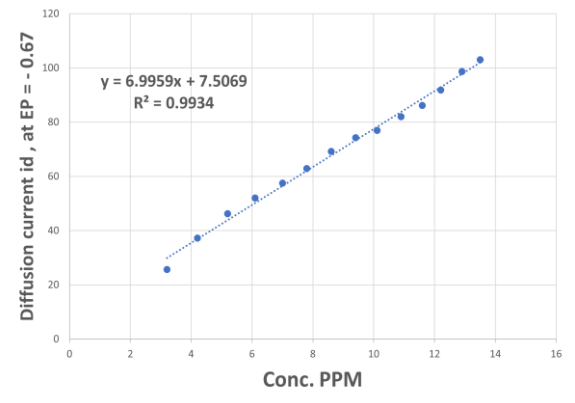

Figure 4: Calibration curve typical for Ceftriaxone

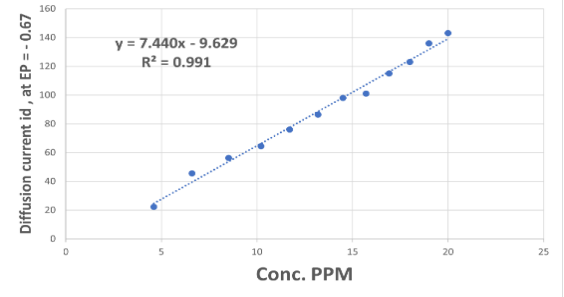

Figure 5: Calibration curve typical for Cefotaxime

3. 3. Results and Discussion

The results suggest the ideal conditions for analysing Ceftriaxone and Cefotaxime are as follows, Table 1.

Table 1: Best conditions for the examination of ceftriaxone and cefotaxime		
Experimental condition	Range	Appropriate conditions
Buffer	citrate buffer Citrate-Phosphate buffer	Citrate-Phosphate buffer
pH	2,4,7,9	2
Supporting Electrolyte	LiCl, KCl, NH4Cl	LiCl, KCl
Solvent	Water-methanol- Acetonitrile	D.W
Mercury drop size	1, 2, 3, 4	4
Temperature, °C	25 °C	25 °C

The accuracy, precision of this method was learned through multiple tests for 10.9 and 10.2 µg.ml-1 laboratory ceftriaxone and cefotaxime samples, the quantity establishes to be 10.749 ± 0.10 and 10.263 ± 0.14 also the relative error ranging from 0.09 to 0.93 and 0.49 to 1.36, the results confirmed appropriate values for standard error of the mean also the confidence limit of the mean, Tables 2 and 3.

Table 2: Analysing ,sample of synthetically produced ceftriaxone								
Initial Conc. µg. ml-1	Computed Current, nA	Determined conc.µg. ml 1	Absolute error	Relative error, %	SD	SEM	%RSD	C.L of the mean
10.9 µg. ml-1	82.8	10.76	0.01	0.09	0.0866	0.049	0.805	10.749±0.2149
	83.3	10.83	0.07	0.65				
	82.0	10.657	-0.10	0.93				
		av. = 10.749						

Table 3: Analysing a sample of synthetically produced cefotaxime.								
Initial Conc. µg. ml-1	Computed Current, nA	Determined conc. µg. ml-1	Absolute error	Relative error,%	SD	SEM	%RSD	C.L of the mean
10.2 µg. ml-1	66.4	10.218	-0.05	0.49	0.154	0.0889	2.376	10.263±0.3823
	68	10.434	0.14	1.36				
	65.8	10.137	-0.14	1.36				
		av. = 10.263						

The appliance for the ceftriaxone estimation in local pharmaceuticals by DPP on DME in an acidic medium with a citrate-phosphate buffer solution at pH of 2 and 1 ml of LiCl, 1 M as a supporting electrolyte; the achieved result illustrates absolute error variety in -0.07 to + 0.07 also the relative error didn't over 0.671%. This method also was employed to determine the amount of ceftriaxone in commercial pharmaceuticals, and the results show that the amount of ceftriaxone in a 1 g vial of ceftriaxone from LDP Pharma Company is between 959 and 972 mg, which is the same as what was originally installed in the product, Table 4.

Table 4: Ceftriaxone commercial drug sample analysis.										
Ceftriaxone vial (for injection), 1g										
Initial Conc. µg. ml-1	Computed Current, nA	Determined conc. µg. ml-1	%Rec	Found in drug	Absolute error	Relative error,%	SD	SEM	%RSD	C.L of the mean
10.8	80.9	10.50	97	972	0.07	0.671	0.0704	0.0406	0.675	10.43± 0.17478
	80.4	10.42	96	964.8	-0.01	0.096				
	80.0	10.36	95.9	959	-0.07	0.671				
		av. = 10.43	96.3	av. =965						

whereas the application for cefotaxime, using DPP on DME in an acidic medium with a citrate-phosphate buffer solution at pH of 2 and 0.5 ml of KCl, 1 M as a supporting electrolyte; the achieved result illustrates absolute error varying in -0.246 to + 0.265 also the relative error didn't over 2.45%, where's the levels of cefotaxime in a vial of 1000 mg, LDP Pharma Company range from 907.5 mg to 951.5 mg, which is only a small fraction of what was intended, Table 5. these results confirmed this developed method have typical precision also repeatability.

Table 5: Cefotaxime commercial drug sample analysis.										
Cefotaxime vial (for injection), 1g										
Initial Conc. µg. ml-1	Computed Current, nA	Determined conc. µg. ml-1	%Rec	Found in drug	Absolute error	Relative error,%	SD	SEM	%RSD	C.L of the mean
11.6	68.7	10.527	90.7	907.5	-0.246	2.28	0.256	0.1478	2.376	10.773 ± 0.6356
	72.5	11.038	95	951.5	0.265	2.45				
	70.4	10.755	92.7	927	-0.018	0.167				
		av. = 10.773	92.8	av. =928.6						

3.1. Limits of detection and quantification

The limit of detection, LOD and limit of quantification, LOQ for ceftriaxone and cefotaxime were computed using a signal-to-noise ratio (S/N) of 3.3 and 10 [12], were found to be 0.84 and 2.802 µg. ml^{-1} for ceftriaxone and 1.4 and 4.66 µg. ml^{-1} for cefotaxime.

3.2. The Number of shared electrons and the value of E½

The Heyrovsky-Ilkovic equation that describes the cathodic reduction at 25 °C, was used to estimate the real value of $E_{1/2}$ and the real number of electrons transferred in a reversible or irreversible electrode process [13,14].

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

This equation illustrated the correlation between the diffusion current with the applied voltage for a reversible or irreversible process, through the relationship generated between Log (i/id-i) versus applied voltage (E), The number of electrons, n looks to be a true number when discussing reversible processes but displays an incomplete number when discussing irreversible processes.

The required reduction electrons, n for ceftriaxone and cefotaxime were calculated. Peaks at -0.67 V show that both ceftriaxone and cefotaxime exhibit increased stability in the steady increase of id. For this reason, in our computation; both Ceftriaxone and Cefotaxime require 5 electrons to undergo reduction and to obtain a straight line, Figures 6 and 7 suggesting that the methoxymine group in these antibiotics are being reduced to methanol [15] and carboxylic acid group electrolysis [16] via an electrochemical mechanism, Figures 8 and 9.

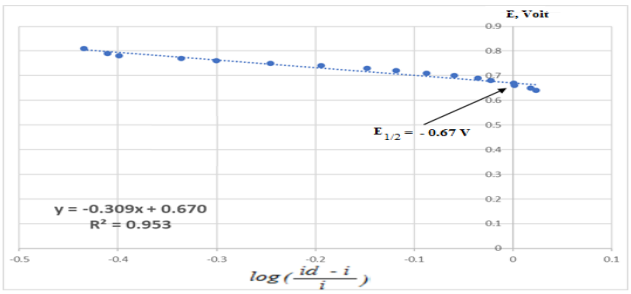

Figure 6: E with log i/id- i difference via Heyrovsky-Ilkovic equation for ceftriaxone.

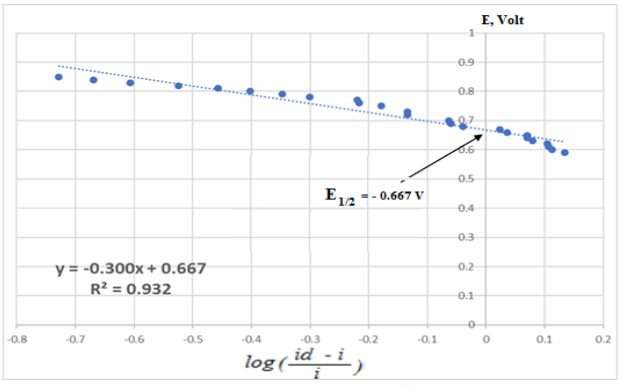

Figure 7: E on the log i/id- i difference with Heyrovsky-Ilkovic equation for cefotaxime.

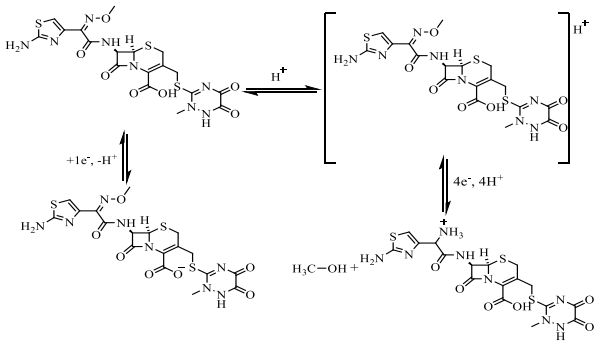

Figure 8: Proposed reduction mechanism for ceftriaxone

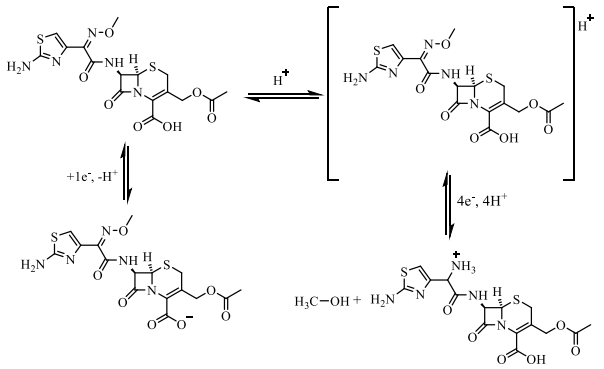

Figure 9: Proposed reduction mechanism for cefotaxime

4. Conclusion

the suggested method's parameters adjusted in just so, a perfect linear correlation (R) was found between peak current and medication concentrations. A commercial combination of ceftriaxone and cefotaxime was used to test the proposed approach, and the results were consistent with the expected values. The proposed method skips the need for time-consuming and laborious sample pre-treatment in favour of a direct, simple, sensitive, and speedy analysis.

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