Quest Journals
Journal of Research in Pharmaceutical Science
Volume 9 ~ Issue 11 (2023) pp: 51-56
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

The Effect of Ceftriaxone on Penicillin in Presence of Phenylalanine

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ABSTRACT: *Under different physical conditions, the effect of ceftriaxone sodium (CEF), a cephalosporin antibiotic, on penicillin PEN, another antibiotic and on phenylalanine PHY and the effect of CEF on (PEN+PHY) in mixed of the three were investigated at PH 7.4 and different temperature using different spectroscopic techniques such as UV-VIS spectroscopy and FT-IR spectroscopy. 5 samples analysis of solutions containing five different concentrations of the ceftriaxone were carried out using UV-VIS spectroscopy and gave a mean correlation coefficient* R^2 *= 0.9953 and molar absorptivity of 5.9 x* 10^3 *L* mol^{-1} cm^{-1} *at* **37**^{o}*C and* λ_{max} *206.6 nm. Different concentrations of PEN and PHY were used to prepare reactions solutions. The reactions solutions were incubated at different temperatures, then the absorbance was measured using UV-Visible spectrophotometer and FT-IR spectroscopy. Values of parameters of binding in terms of binding constants for CEF+PEN, CEF+PHY, PEN+PHY were measured. For CEF+PEN it was found to be (*4×10^3 *at* **37**^{o}*C), for CEF+PHY was found to be (*7×10^3 *at* **37**^{o}*C), for PEN+PHY was found to be (*1×10^4 *at* **37**^{o}*C) and for CEF+PEN with direct addition of PHY was found to be (*1.3×10^4 *at* **37**^{o}*C) similar to that for CEF+PEN when PHY was added after 30 minutes. The (FT- IR) observed spectral changes indicated the formation of peptide-bond between ceftriaxone and penicillin.*

KEYWORDS: *phenylalanine; penicillin; ceftriaxone; correlation coefficient.*

R*eceived 04 Nov., 2023;* ***R****evised 14 Nov., 2023;* ***A****ccepted 16 Nov., 2023 © The author(s) 2023.*
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I. INTRODUCTION

The beta-lactam antibiotics have been discovered and used by mankind for over 70 years. Regardless of this age, they still provide health to the world population. Sales of these remarkable compounds have reached over $20 billion dollars per year [1]. Ceftriaxone (CEF) and penicillin (PEN) are types of these antibiotics. The ceftriaxone widespread use and the transport of ceftriaxone through the blood-brain barrier, make it necessary to study the structural changes of ceftriaxone -protein complexes to understand the biological effects and functions of ceftriaxone in the body [2]. In recent years, many investigations into the binding of drugs were carried out. Pan et al. (2012) did Biophysical studied on the effect of ceftriaxone sodium (CS) on bovine serum albumin (BSA) using spectroscopic methods, they indicate that CS binds BSA mainly through H-bonding or Van der Waals forces, leading to secondary structural changes and there is high possibility that energy transferred from BSA to CS [3].

Lavany et al. (2015) studied the role of CH-O interactions and its effect on stability and specificity of penicillin binding proteins (PBPs), they found that all the residues located in the binding pockets of penicillin binding proteins are due to CH…O interactions. [4]. Another study, effect of Glial fibrillary acidic protein (GFAP) on ceftriaxone and phenytoin: SRCD and molecular docking and dynamic simulation which done by Ruzza et.al, (2016) and they observed that ceftriaxone and phenytoin interact directly with GFAP increasing the content of α-helical structure [5]. In addition Rhman et al., (2016) consider the effect of temperature and salts on the interaction between cetyltrimethyl ammonium bromide (CTAB) and ceftriaxone sodium trihydrate drug (CFT), they illustrate that the interaction between CFT and CTAB caused a change of critical micelle concentrations $C^*$of CTAB values which become more than $C^*$of pure CTAB in aqueous solution and C^* values for (CFT+CTAB) mixed system decreases by the presence of salts as compared to aqueous medium [6]. Ceftriaxone, as a possible ligand, has many studies of its bindings but it was not studied in detail upon its binding reaction with penicillin. In this study, we will investigate the effect of ceftriaxone on penicillin in presence of phenylalanine by using different spectroscopic techniques such as FT-IR spectroscopy and UV-VIS spectroscopy. The reaction mechanism expected is the interaction between carboxyl group and amino group to form peptide bond.

II. MATERIALS

All the chemicals used were of analytical grades. The distilled water was used throughout the study. Pure Ceftriaxone solid salt CEF, Penicillin -G Potassium (BI Biological Indusries) PEN, phenylalanine salt, sodium chloride (NaCl) (Sigma, USA). sodium monophosphate (NaH_2PO_4) salt (Sigma, USA), sodium diphosphate (Na_2HPO_4) salt (Sigma, USA), Buffer solutions pH 7.4 at unit internal were prepared from (0.2M) monobasic sodium phosphate (NaH_2PO_4), (0.2M) dibasic sodium phosphate ($Na_2H\ PO_4$) and 9.8 g Sodium Chloride.

III. METHADOLOGY

The solutions of CEF (5. 10, 15, 20, 25μM) were prepared in phosphate buffered saline (PBS). The solutions of PEN (50. 100, 150, 200. 250μM) and PHE (50, 100, 150, 200, 250μM) were prepared in PBS. The spectrophotometric studies were carried out with UV-VIS spectrophotometer –Shimadzu-equipped with 1.0-cm quartz cells. Data were recorded at maximum wave length 206.6 nm, FT-IR spectrophotometer -Bruker alpha- was used.

Table 1: Reaction samples solutions of ceftriaxone with different concentration of penicillin.

Group1	Group2	Group3	Group4	Group5
$C_1 + P_1$	$C_2 + P_1$	$C_3 + P_1$	$C_4 + P_1$	$C_5 + P_1$
$C_1 + P_2$	$C_2 + P_2$	$C_3 + P_2$	$C_4 + P_2$	$C_5 + P_2$
$C_1 + P_3$	$C_2 + P_3$	$C_3 + P_3$	$C_4 + P_3$	$C_5 + P_3$
$C_1 + P_4$	$C_2 + P_4$	$C_3 + P_4$	$C_4 + P_4$	$C_5 + P_4$
$C_1 + P_5$	$C_2 + P_5$	$C_3 + P_5$	$C_4 + P_5$	$C_5 + P_5$

25 samples of reactions solutions of different concentration were incubated at (25°C, 37°C, 50°C) for three hours each, and then they were monitored using the UV–Visible spectrophotometer and FT_IR spectrophotometer. Either, solutions of CEF were prepared at 0.01, 0.10, 0.50, 1.0, 5.0, 10.0, 15.0, 20.0 and 25.0μM concentrations named as C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9. Then samples reaction solutions were prepared by using 1.5 ml of 250.0μM penicillin solution and 1.5 ml of C solutions each. Then, 9 samples of reaction solutions incubated at 37 °C to complete the reaction. At the end the samples reaction solutions monitored by using UV–Visible spectrophotometer at the 206.6 nm and also evaluated by FT_IR spectrophotometer.

For reaction of CEF with PHE, the five solutions samples were prepared Table 2

Table 2: Reaction samples solutions of ceftriaxone with different concentration of phenylalanine.

C + Ph1	1.5 ml CEF 20μM + 1.5ml PHY 50μM
C + Ph2	1.5 ml CEF 20μM + 1.5ml PHY 100μM
C + Ph3	1.5 ml CEF 20μM + 1.5ml PHY **1**50μM
C + Ph4	1.5 ml CEF 20μM + 1.5ml PHY 200μM
C + Ph5	1.5 ml CEF 20μM + 1.5ml PHY **2**50μM

The five samples solutions were incubated in bathwater at (37◦C, 50◦C) for three hours each. The solutions were incubated in a bathwater at 37◦C for three hours then the absorbance was measured using UV –Visible spectrophotometer.

For reaction of PEN with PHE, five samples of the following solutions were prepared Table 3.

Table 3: Penicillin samples solutions Reaction different concentration of phenylalanine.

Pen + Ph1	1.5 ml PEN 200μM + 1.5ml PHY 50μM
Pen +Ph2	1.5 ml PEN 200μM + 1.5ml PHY **10**0μM
Pen +Ph3	1.5 ml PEN 200μM + 1.5ml PHY **1**50μM
Pen +Ph4	1.5 ml PEN 200μM + 1.5ml PHY **2**00μM
Pen +Ph5	1.5 ml PEN 200μM + 1.5ml PHY **2**50μM

The solutions were incubated in bathwater at (37◦C, 50◦C) for three hours each, then the absorbance were measured using UV-Visible spectrophotometer.

At the end CER with PEN were mixed then PHY was added by two ways, directly and after thirty minutes of mixing, the following samples solutions were prepared:

Table 4: phenylalanine samples solutions reaction with a mixture of Penicillin and ceftriaxone directly and after thirty minutes

Addition directly	Addition after 30 minutes
(1.5 ml CEF +1.5 ml PEN) + 1.5 ml PHY	(1.5 ml CEF +1.5 ml PEN) + 1.5 ml PHY
(20µM +200µM) +50µM	(20µM +200µM) +50µM
(20µM +200µM) +100µM	(20µM +200µM) +100µM
(20µM +200µM) +150µM	(20µM +200µM) +150µM
(20µM +200µM) +200µM	(20µM +200µM) +200µM
(20µM +200µM) +250µM	(20µM +200µM) +250µM

IV. RESULTS AND DISCUSSION

Ethiraj et. al., (2014) studied ceftriaxone spectroscopy found a linear relationship between absorbance at maximum wave length and various CEF concentration.

The linearity graph obeyed Beer's law and described by liner equation $y = ax + c$. The correlation coefficient R^2 was very close to 1 [7]. This result is compatible with our result of CEF absorbance that determined by UV-Visible Spectrophotometer. A linear relationship between absorbance and various CEF concentration at range from 5µM to25µM at maximum wave length 206.6 nm was found.

The correlation coefficient R^2 was 0.9953 as shown in figure1 and Table 4, the results indicated that the maximum absorbance of all different concentration was at 206.6 nm as it appeared.

Table .5: Different CEF concentrations absorbance

ceftriaxone	Absorbance
5µM	0.7170
10µM	0.8900
15µM	1.0650
20µM	1.1930
25µM	1.3320

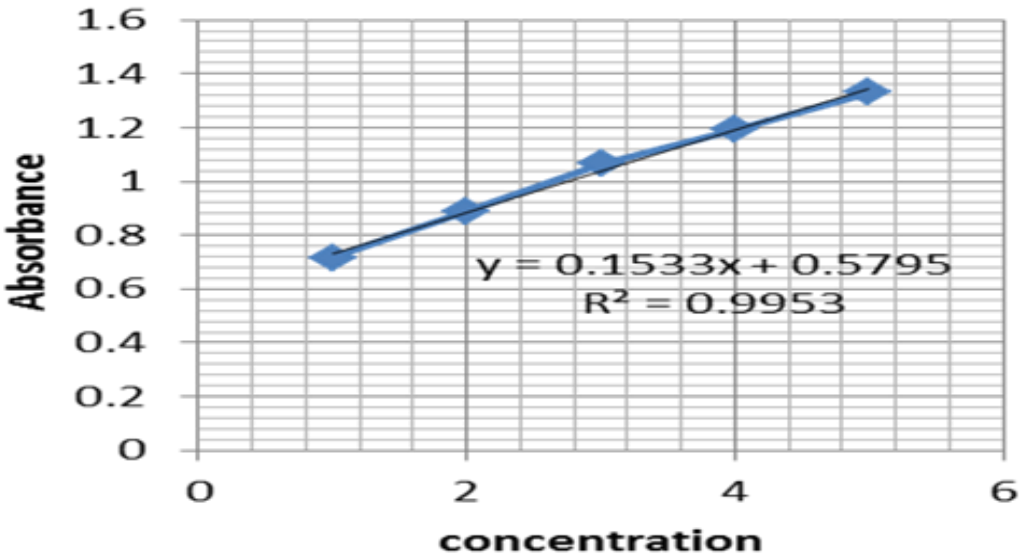

Figure .1: relationship between absorbance at maximum wave length and various CEF concentration.

Table. 6: Reactions of mixture of CEF and PEN results Absorbance at different temperature.

	CEF] / PEN	5µM	10µM	15µM	20µM	25µM
Absorbance at 25˚C	50 µM	1.188	1.113	1.202	1.204	1.274
	100 µM	1.58	1.431	1.557	1.563	1.630
	150 µM	1.856	1.765	1.860	1.900	1.940
	200 µM	2.165	2.114	2.223	2.241	2.312
	250 µM	2.482	2.515	2.571	2.571	2.709
Absorbance at 37˚C	50 µM	1.005	1.081	1.151	1.227	1.209

	100 µM	1.407	1.468	1.500	1.589	1.565
	150 µM	1.808	1.806	1.974	1.849	1.09
	200 µM	2.346	1.989	2.16	2.173	2.270
	250 µM	2.422	2.451	2.270	2.552	2.552
Absorbance at 50˚C	50 µM	1.167	1.041	1.064	1.133	1.178
	100 µM	1.458	1.421	1.517	1.468	1.524
	150 µM	1.762	1.747	1.817	1.865	1.872
	200 µM	2.157	2.181	2.173	2.205	2.260
	250 µM	2.498	2.395	2.422	2.533	2.571

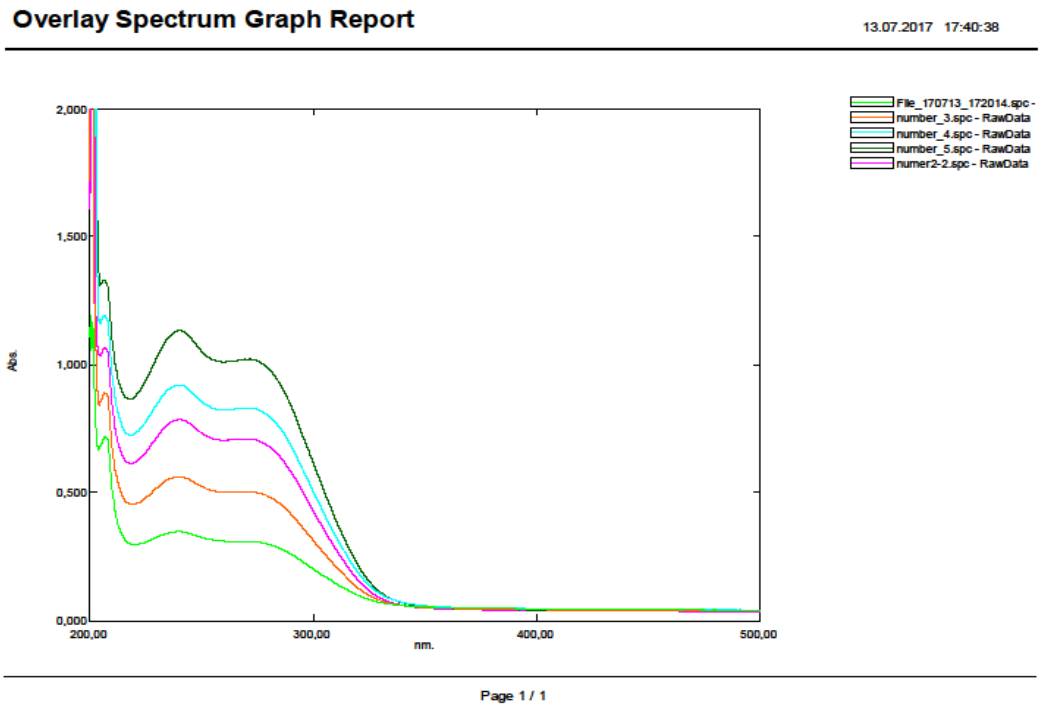

Figure .2 Different CEF concentrations absorbance.

The UV-Visible spectrophotometric analysis of interaction between CEF and PEN, CEF and PHY and the interaction between mixer of PEN and CEF with PHY found dependent on the concentration at different temperature. In our results the increase in absorption is almost directly proportional to the reactants concentration which indicated in Tables (7 to 9) and illustrated by the plot of absorbance versus the concentration of reactants at maximum wave length which are fitting to linear equation and obeyed the Beer's law.

Table 7: Absorbance of reactions of mixture of CEF with PEN at different temperature.

	CEF / PEN	20µM
Absorbance at 37˚C	50µM	1.061
	100µM	1.244
	150µM	1.418
	200µM	1.708
	250µM	1.785
Absorbance at 50˚C	50µM	1.044
	100µM	1.192
	150µM	1.362
	200µM	1.544
	250µM	1.678

Table 8: Absorbance of reactions of mixture of CEF with PHY at different temperature.

	CEF / [PHY	200µM
Absorbance at 37°C	50µM	1.741
	100µM	2.128
	150µM	2.275
	200µM	2.382
	250µM	2.524
Absorbance at 50°C	50µM	1.945
	100µM	2.142
	150µM	2.29
	200µM	2.422
	250µM	2.516

Table 9: Absorbance of reactions of mixture of PEN and CEF with PHY added directly and with PHY added After 30 minutes at 37°C

	CEF +PEN / PHY	*20µM +200µM*
Absorbance at 37°C when PHY added directly	50µM	1.701
	100µM	1.817
	150µM	1.917
	200µM	2.05
	250µM	2.107
Absorbance at 37°C when PHY added After 30 minutes	50µM	1.704
	100µM	1.849
	150µM	1.94
	200µM	2.081
	250µM	2.173

It was easy to notice that when concentration of CEF was 20µM they gave the best results, this concentration of CEF 20µM was used to study the binding constant of CEF with different concentration of PEN at 37◦C, 50◦C and with different concentration of PHY at 37◦C, 50◦C. The result illustrated the increasing of binding constant between CEF and PHY from 7×10^3 at 37◦C to 8×10^3 at 50 ◦C. The binding constant between the mixture of PEN and CEF with PHY was 1.3×10^4 even it added directly or after 30 mints.

The concentration of CEF 20µM was either used to study the FT-IR spectroscopy of interaction of CEF with PEN at 25◦C, 37◦C. FT-IR stereoscopy results indicated evidence interaction between CEF and PEN. The disappeared of primary amine pikes of CEF at (3100 cm^{-1} – 3500 cm^{-1}) which must be two sharp pikes [8] and appeared of abroad single pike in this region illustrated the excitant of peptide bond. This indicated by presence of C=O stretching and N-H group which found in region 1630-1640 cm^{-1} [8]. All presence pikes effected by the concentration and have a small shifted. Hsih et. al., (2015) found that the PEN and CEF acted synergistically. They informed that the reason for this is unclear (Hsieh et. al., 2015). However, our results explained that it was due to the reaction between PEN and CEF to form peptide bond.

V. CONCLUSION

In conclusion from the above results of analysis of the effect of CEF on PEN and PHY by UV-Visible Spectrophotometer and FT-IR spectrophotometers techniques there were avidness for their binding. From UV-Visible spectroscopy results, the absorbance was directly increasing by increasing of the concentration of the reactants and by the increasing of temperature and fitting to linear equation and obeyed the Bear 'Low. The binding constant K_p of (CEF+PEN) was registered 4×10^3 at 37◦C and 3×10^3 at 50 ◦C, The binding constant K_p of (CEF+PHY) is registered 7×10^3 at 37◦C and 8×10^3 at 50 ◦C, The binding constant K_p of (PEN+PHY) was registered 1×10^4 at 37◦C and 1.4×10^4 at 50 ◦C, .We notice when PHY add to (CEF+PEN), the binding constant Kp was 1.3×10^4 even it added directly or after 30 mints.

In addition, the results of FT-IR spectroscopy illustrate that the primary amine NH_2 of CEF which appear as two pikes at 3100-3500 cm^{-1} did not appear and instead there was a brad pike of secondary amid at 3100-3400 cm^{-1} which was very sensitive to the strength of hydrogen bond [10], this was more indicated by presence of stretching vibration of C=O and C-N groups which found in the range between 1630-1640 cm^{-1}.

From all that we suggested that the reaction between amino group of CEF and an acidic group of PEN may be taking place and illustrates a present of peptide bond which contains the secondary amine. Further studies are needed to investigate the effect of this interaction on cell culture

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