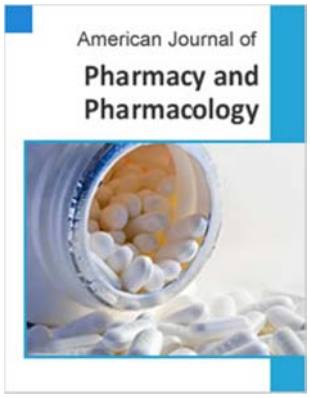
American Journal of Pharmacy and Pharmacology
2017; 4(2): 5-9
http://www.aascit.org/journal/ajpp
ISSN: 2375-3900

AASCIT American Association for Science and Technology



Keyword
Ceftriaxone,
Phenylalanine,
DNA Interaction

Received: March 28, 2017
Accepted: April 18, 2017
Published: June 13, 2017

Interactions of Ceftriaxone with Phenylalanine and CT-DNA

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Citation

Hakan Sezgin Sayiner, Fadhila Othman, Aisha Abdelsalam, Cengiz Baloglu, Yasemin Celik Altunoglu, Fatma Kandemirli. Interactions of Ceftriaxone with Phenylalanine and CT-DNA. *American Journal of Pharmacy and Pharmacology*. Vol. 4, No. 2, 2017, pp. 5-9.

Abstract

The interaction of ceftriaxone and phenylalanine with different temperatures and incubation times were determined by Ultraviolet-Visible spectrophotometer (UV) and also interaction between ceftriaxone and Calf-Thymus Deoxyribonucleic acid (CT-DNA) by UV absorbance. Values of binding parameters for ceftriaxone – phenylalanine inreraction in terms of binding constant were found to be 2.4×10^4, 2.8×10^4, 1.8×10^4, 2.0×10^4, 3.0×10^5 for 5 minutes incubation time and pH:7. Binding parameter values for Ceftriaxone and CT-DNA at 25°C, 30°C, 40°C and 50°C were found as 3.3×10^4, 8×10^3, 6×10^3 and 2.2×10^4 respectively.

1. Introduction

Ceftriaxone is a third-generation cephalosporin, which is classified as beta-lactam antibiotics. It works on Gram-positive bacteria and resistant Gram-negative bacteria, and this is the thing that separates it from the second generation cephalosporin and it is very useful for most bacterial infections. The common name is Rocephin and it is resistant to most infections, including pneumonia, inflammation of the skin, and internal inflammation of the abdomen. They give it before surgeries to avoid inflammation later and given intravenous and intramuscular [1]. Isolation of cephalosporin compounds for the first time were achieved from *Cephalosporium acrimonious* in Sardinia in 1948 by an Italian scientist Giuseppe Brotzu. Ceftriaxone was discovered beginning in the 1980s by Hoffmann-La Roche. Ceftriaxone is an antibiotic of the third-generation cephalosporin and it prevents the formation of proteins that are necessary for the installation of the cell wall of bacteria [2]. Ceftriaxone antibiotics are used to treat microbes that are not resistant to many other antibiotics [3]. Ceftriaxone is available by intramuscular injection or infusion [4]. It should not be used to ease the solution containing calcium and also solvents containing calcium should not be injected into the veins [5]. Ceftriaxone adverse effects are a change in the white blood cells which resulting skin rashes and diarrhea [6]. Ceftriaxone should not be used for those who are allergic to it and allergic to penicillin. In addition, ceftriaxone should not be used for newborn babies, especially prematurely births. Because it causes the isolation of bilirubin from albumin, which causes inflammation in the brain.

Ceftriaxone was observed that might cause abnormalities in the fetus, so it must not be given to pregnant women [7].

Ceftriaxone is excreted low concentrations in breast milk so it is not expected to cause damage in infants. There are no studies which proving of dangerous ceftriaxone but the sensitivity to ceftriaxone is more common in old patients than adolescent [8]. Ceftriaxone is given by intramuscular or intravenous way and cannot be given by mouth. Ceftriaxone penetrates the tissues and body fluids such as liquid spinal cord well to address the central nervous system infections [9]. Ceftriaxone interacts with many antibiotics, especially aminoglycosides like gentamicin, tobramycin and also live bacterial vaccines and parenteral solutions containing calcium [10].

2. Material and Methods

2.1. Interaction Ceftriaxone with Phenylalanine

The stock solution of ceftriaxone was prepared by dissolving 0.00554 gr of ceftriaxone in 10 ml of Tris - HCl at Ph 7 to obtain 1 M ceftriaxone solution. The stock solution of phenylalanine was prepared by dissolving 0.00165 gr of phenylalanine in 10 ml of Tris - HCl at Ph 7 to obtain 1 M phenylalanine solution

One molar of Tris - HCl was prepared by dissolving 121.1gr of Tris base in 700 ml of distilled water (dH_2O) and desired Ph was calibrated to 7.5 with concentrated HCl and then completed to 1 L with distilled water, filtered with 0.5 µ filter in diameter and autoclaved. The solution was stored at room temperature. A dilution solution of Tris-HCl was prepared from 10 ml stock solution and 90 ml distilled water [11].

Extension coefficient was calculated by using Ultraviolet-Visible spectrophotometer (UV) (Multiscan Go, Thermo Fischer, USA). The Beer's Law states that the constant molar absorption depends on the concentrations of dissolved substances in the solution at a specific wavelength. Extinction coefficients are the common name of molar absorptive. The units of molar absorptivity are canceled with units of concentration and light path measurement [12].

2.2. Interaction Ceftriaxone with Calf Thymus-DNA (CT-DNA)

The CT-DNA that is used in the experiment was purchased from GE Healthcare Life of Sciences. About 0.003 gr of CT-DNA was dissolved in 3 ml of Tris-HCl (Ph7.4). The UV ratio of CT-DNA solutions was absorbed at 260 and 280 nm wavelenght. The concentration of the nucleotide was determined by UV absorption spectroscopy using the molar absorption coefficient ($\varepsilon = 6600 M^{-1}cm^{-1}$) at 260nm.

3. Result and Discussion

Extension coefficient of ceftriaxone was calculated according to the equation of $A=\varepsilon c\ell$, where ε is the molar absorptivity (the coefficient of that material), c is the concentration amount of those species, ℓ is the path length. Higher absorption was determined at 234 nm 278 nm wavelength and. In Table 1 the extinction coefficient for the temperature (at 25°C, 30°C for different concentrations of ceftriaxone at pH:3, pH:7 and pH:9 the absorption rates of each by Ultraviolet-Visible spectrophotometer (UV) for 234 nm were shown in Table 1.

Table 1. *The extinction coefficient of ceftriaxone for pH:3, pH:7 and pH:9 for different temperatures.*

Temperature	Extinction coefficient $M^{-1}cm^{-1}$		
	Ph:3	**Ph:7**	**Ph:9**
25°C	18647	18237	19335
30°C	19552	17714	19000
40°C	18525	18920	19492
50°C	19864	18725	18900
60°C	19394	19589	19725

3.1. Interaction Between Ceftriaxone and Phenylalanine

Five samples containing the fixed concentration of ceftriaxone (10 µM) and variable concentrations of phenylalanine (100-150-200-250-300 µM) diluted with Tris - HCl (Ph 7), incubated 5 and 20 minutes at different temperatures (25°C-30°C-40°C-50°C-60°C) and measured by Ultraviolet-Visible spectrophotometer (UV).

The absorption spectra of the ceftriaxone in the absence and presence of phenylalanine is illustrated for pH 3 and 30°C in Figure 1.

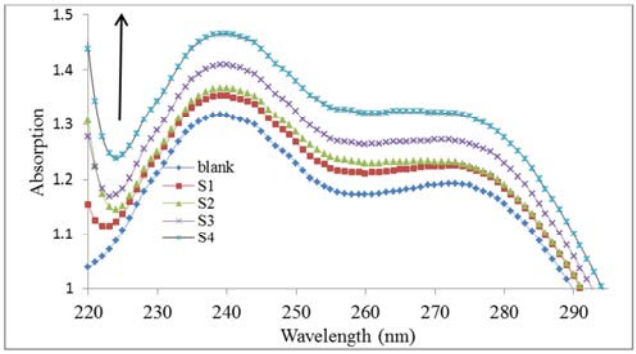

Figure 1. *Interaction ceftriaxone with phenylalanine at PH:3, 30°C for 5 minutes. (----) shows the absorption of ceftriaxone alone and (_____) shows the absorption of different concentrations of phenylalanine with ceftriaxone.*

Binding constant values were calculated with the following formula.

$$\frac{[C]}{(\varepsilon_a - \varepsilon_f)} = \frac{[C]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \quad [13]$$

[C] is the concentration of phenylalanine/CT-DNA in base pairs. ε_a, ε_f, and ε_b are free and bound ceftriaxone/ CT-DNA extinction coefficients. In particular, ε_f was determined by a calibration curve of ceftriaxone in aqueous solution, following Beer's law. ε_a was determined as the ratio between

the measured absorbance and ceftriaxone concentration, Aobs/ [ceftriaxone]. A plot of [C]/(ε_a-ε_f) vs [C] gives a slope of 1/(ε_b-ε_f) and a y-intercept equal to 1/Kb (ε_b-ε_f); Kb is the ratio of the slope to the y-intercept [42].

Kb values for the interaction of between ceftriaxone and phenylalanine at pH:3, pH:7 and pH:9 in different temperatures and incubation times are given in Table 2.

As seen from the Table 2 the binding constant between ceftriaoxone and phenylalanine decreases with increasing pH. The binding constants for pH 3, pH 7 and pH 9 for 5 minutes incubation time at 25 ° C are 3.7×10^4, 2.4×10^4 and 1.7×10^5, respectively.

Table 2. *Kb values for the interaction of ceftriaxone with phenylalanine at pH:3, pH:7 and pH:9 in different temperatures and incubation times.*

Temp	pH=3		pH=7		pH=9	
	5 min	20 min	5 min	20 min	5 min	20 min
25°C	3.7×10^4	1.0×10^5	2.4×10^4	3.04×10^4	1.7×10^5	1.16×10^5
30°C	5.6×10^4	1.3×10^5	2.8×10^4	2.04×10^4	9.8×10^4	1.5×10^5
40°C	4.5×10^4	5.8×10^4	1.8×10^4	4.3×10^4	1.3×10^6	1.2×10^5
50°C	5.7×10^4	6.9×10^4	2.0×10^4	6.1×10^4	1.18×10^5	1.3×10^5
60°C	4.0×10^4	9.1×10^4	3.0×10^5	6.0×10^3	1.18×10^5	7.9×10^4

The binding constant between ceftriaoxone and phenylalanine at pH:3 are in the range 3.7×10^4- 5.7×10^4 for 5 minutes incubation time and for pH:7 are in the range 2.4×10^4-3×10^5, for pH:9 are in the range. 1.16×10^{-5}-7.9×10^4.

3.2. Interaction Ceftriaxone with Calf Thymus-DNA (CT-DNA)

The interactions between ceftriaxone and CT-DNA at Ph7 in different temperatures were performed.

The effect of CT-DNA on ceftriaxone by Ultraviolet-Visible spectrophotometer (UV)at temperatures 25°C, 30°C, 40°C, 50°C, 60°C for 5 minutes in Ph7 were performed. As seen from the Figure 2-5, an increase in concentration of CT-DNA raised the absorbance values.

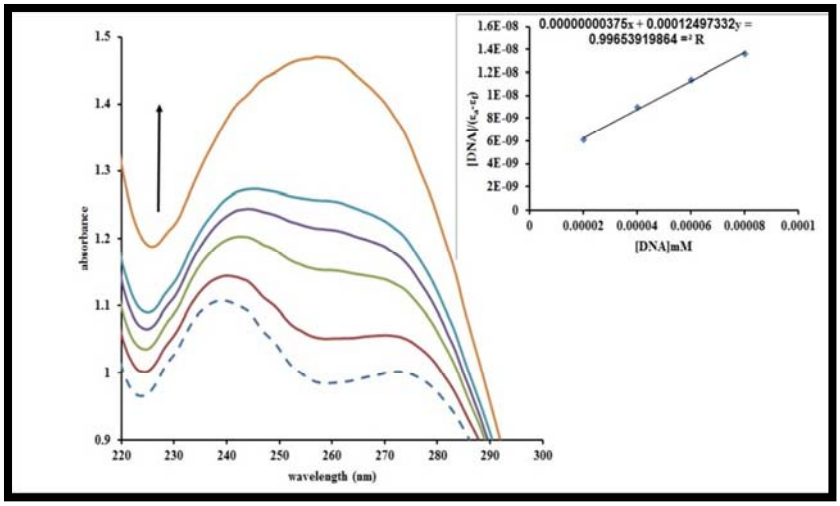

Figure 2. *Interaction between ceftriaxone and CT-DNA at 25°C for 5 minutes.*

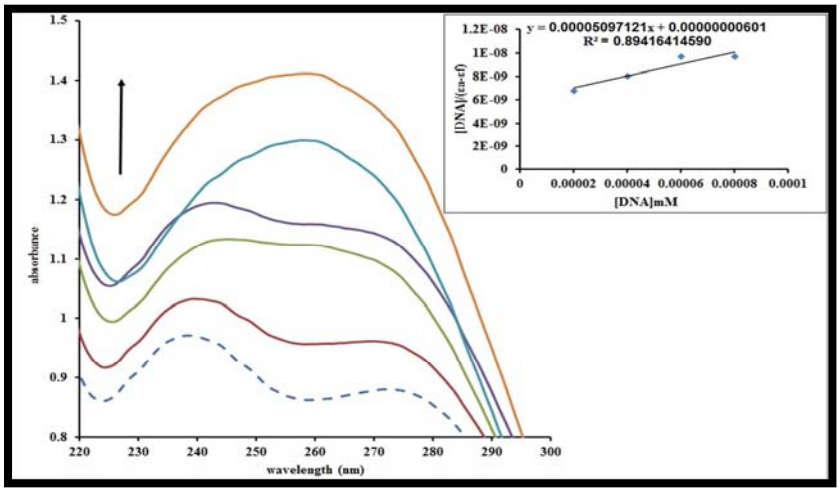

Figure 3. *Interaction between ceftriaxone and CT-DNA at 30° for 5 minutes.*

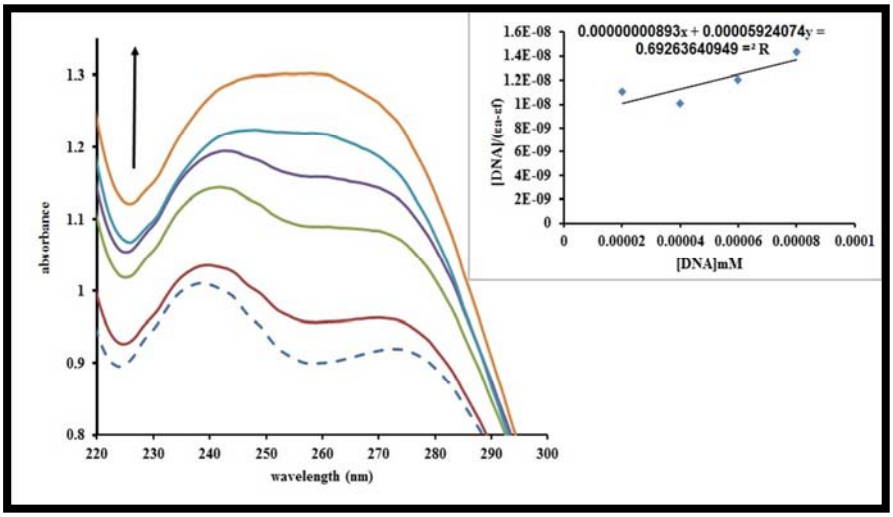

Figure 4. *Interaction between ceftriaxone and CT-DNA at 40°C for 5 minutes.*

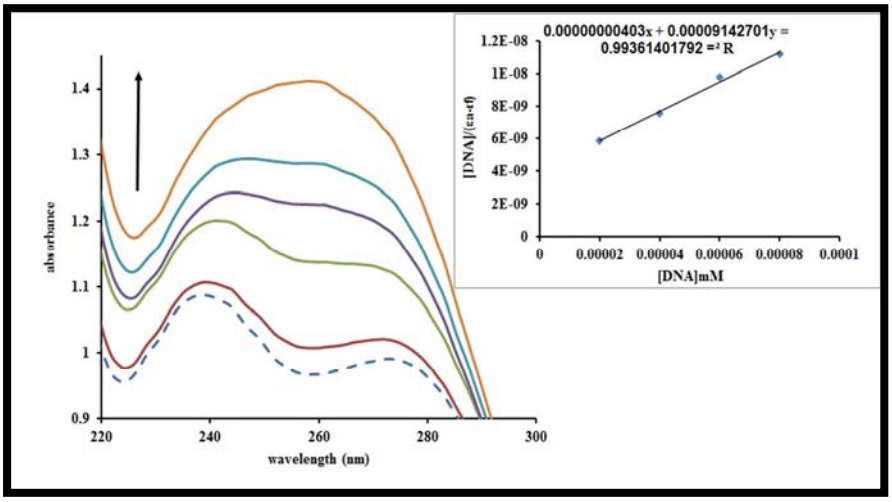

Figure 5. *Interaction between ceftriaxone and CT-DNA at 50°C for 5 minutes.*

From past outcomes follows that the higher concentrations of CT-DNA with ceftriaxone have increased the absorbance rates at different temperatures, also this change the Kb values which are presented in the Table 3

Table 3. *Kb values for the interaction of ceftriaxone with CT-DNA.*

Temperature	Kb values
25°C	3.3×10^4
30°C	8×10^3
40°C	6×10^3
50°C	2.2×10^4

The Kb values of ceftriaxone at 25°C, 30°C, 40°C and 50°C are 3.3×10^4, at was 8×10^3, at was 6×10^3 and 2.2×10^4 respectively..

Kong et al. (2012) evaluated the interaction between insulin and CT-DNA by fluorescence and circular dichroism spectroscopies at Ph7.4. The results indicated that the greater concentration amounts of CT-DNA provide an increase in absorption rates [14]. This is consistent with our results and show that the increase in concentration of CT-DNA at pH 7 causes an increase in the interaction of ceftriaxone and phenyl alanine.

Kong et al. (2011) evaluated the interaction between polymyxin B (PMB) and DNA. According to the results, the greater concentration of polymyxin B causes the absorption increase with DNA [15]. This is consistent with the result of the interaction between ceftriaxone and CT-DNA at Ph7 where the greater concentration of CT-DNA with ceftriaxone causes an increase in the absorption ratios by Ultraviolet-Visible spectrophotometer (UV).

Pradeep et al. (2016) studied with the targeted anticancer ruthenium (II) complex units containing bpy (2,2′-bipyridine), phen (1,10-phenanthroline) or 2,9-dmp (2,9-dimethyl 1,10-phenanthroline) and anchored on a branched polyethyleneimine molecule (Rul-BPEI). The interaction between this complex with CT-DNA was investigated by absorption spectroscopy [16]. The interaction was consistent with the interaction between ceftriaxone and CT-DNA that was investigated in our study. Mechanisms between ceftriaxone and phenylalanine were studied in previous study and reported that stepwise mechanism proceeded through a tetrahedral intermediate and a concerted mechanism by using semi-empirical-PM6 method [17].

4. Conclusion

To sum up, reactions of ceftriaxone and phenylalanine interactions are incremental. Ceftriaxone was examined with CT-DNA to check its interaction with CT-DNA by using ultraviolet-visible spectrophotometer technique and found sufficient evidences for its binding mode. The Kb values of ceftriaxone with phenylalanine at 25°C was 2.4×10^4, at 30°C was 2.8×10^4, at 40°C was 1.8×10^4, at 50°C was 2.04×10^4 and at 60°C at 3×10^5 for 5 minutes, and for 20 minutes at 25°C was 3.04×10^4, at 30°C was 2.04×10^4, at 40°C was 4.3×10^4, at 50°C was 6.1×10^4 and at 60°C was 3.04×10^4. The Kb values of ceftriaxone in CT-DNA at 25°C was 3.3×10^4, at 30°C was 8×10^3, at 40°C was 6×10^3 and at 50°C was 2.2×10^4.

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