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Voltammetric and theoretical study of the interaction of ceftriaxone with phenylalanine

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The interaction between ceftriaxone and phenylalanine (PA) was investigated by cyclic voltammetry and quantum chemical study using DFT (density functional theory) method. The study was carried out in phosphate buffer solution (PBS) at pH 7.0 (which was also used as the supporting electrolyte) by directly dissolving it in twice distilled water. The voltammetric study of ceftriaxone showed well expressed redox peaks at 0.090 V on a GCE in phosphate buffer of pH 7.0 at 50 mVs^{-1}. The cathodic peak currents were linear with different scan rates from 25 to 275 mVs^{-1} and the correlation coefficient was found to be 0.971 9 and 0.9592 for ceftriaxone and ceftriaxone-PA systems, respectively in the potential range of 0.8-(-0.2) V. The electron transfer rate constant (k_s) was calculated for the reduction of ceftriaxone and ceftriaxone-PA interactions as 2.031 and 4.831 s^{-1}, respectively. After the addition of PA to the ceftriaxone solution, the redox binding constant was obtained as $K = 1.32\times10^3\ M^{-1}$ for ceftriaxone-PA interaction, and quantum chemical calculations were performed for ceftriaxone and ceftriaxone-PA complex by the B3LYP method.

Keywords: Ceftriaxone, Phenylalanine, DFT (density functional theory) method, Cyclic Voltammetry (CV)

INTRODUCTION

The bactericidal activity of ceftriaxone antibiotic belonging to the third generation of cephalosporin is due to its inhibition of the synthesis of the bacterial cell wall [1]. Ceftriaxone shows a broad spectrum of activity against Gram-negative and Gram-positive pathogens; it is effective against a wide range of infections such as skin and skin structure infections, pelvic inflammatory disease, meningitis, uncomplicated gonorrhea, intra-abdominal infections [2].

Reynold suggested that ceftriaxone, when dissolved in balanced salt solutions, enters normal rat brain with a PS product similar to that of mannitol and that the penetration of "C-labeled ceftriaxone from the perfusate into the substance of the brain can be inhibited by the weak acid transport-system blocker probe [3].

Abu Teir *et al.* [4] studied the interaction between human serum albumin and ceftriaxone under physiological conditions by UV absorption and fluorescence spectroscopy and reported that ceftriaxone showed a strong ability to quench the intrinsic fluorescence of human serum albumin and estimated the binding constant (k) as $K=1.02\times 10^3\ M^{-1}$ at 298 K.

Song *et al.* [5] performed electrochemical synthesis of gold nanoparticles on the surface of glassy carbon electrode and prepared GNPs in aqueous solution with ceftriaxone as an innocuous stabilizing agent and reported that the modified electrode has excellent repeatability. Sayiner *et al.* [6] studied the concerted and stepwise mechanisms for the peptide bond formation of the carboxyl group of ceftriaxone with the amino group of phenylalanine and the carboxyl group of phenylalanine with the amino group of ceftriaxone with the semi-empirical PM6 method. Jabbar *et al.* [7] studied the interaction of riboflavin with cadmium (Cd) in aqueous media by cyclic voltammetry, differential pulse anodic stripping voltammetry and chronocoulometry.

Due to their high sensitivity and selectivity, voltammetric methods have been successfully used to investigation of the redox behavior of various biological compounds. Electrochemical techniques are excellent diagnostic techniques that have been used for the determination of biological properties of electroactive species and organic molecules, including amino acids. Amino acids and proteins are important electroactive species in all basic biological processes in the cell [8].

Phenylalanine (2-amino-3-phenylpropanoic acid), a component of many central nervous system neuropeptides, is an essential amino acid for humans. It is widely used as a food or feed additive in infusion fluids or for chemical synthesis of pharmaceutically active compounds [9].

The binding ability of drug with protein is an important subject in life process that helps us to understand the absorption, transport, metabolism

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and the target molecules of the drugs at the cellular level. Therefore, this interaction will significantly affect the apparent distribution volume of the drugs and also affect the elimination rate of drugs in most cases. On the other hand, clarification of the nature of the interaction between the drug molecule and the target protein is a key process in the development of new drugs, which can contribute to the elucidation of the interaction of drug with biomolecules [10-11].

There are a variety of techniques currently available for the measurement of binding constants for redox active species. Compared with these methods, cyclic voltammetry which is a frequently used electrochemical assay, is simple, low-cost, easily implemented, and has fast response [12-16].

To our knowledge, there is no scientific report on the study of ceftriaxone interaction with phenylalanine, the electrochemical behavior, or their quantum chemical study. In this paper, quantum chemical studies were performed for the cationic form of ceftriaxone, the protonated form of ceftriaxone, the complex form of ceftriaxone with phenylalanine using the density functional theory (DFT), within a standard Gaussian 09 (Revision B.05) [17]. The lowest unoccupied molecular orbital energy (E_{LUMO}), the highest occupied molecular orbital energy (E_{HOMO}), the energy gap between the energy of HOMO and LUMO, hardness, softness, polarizabilities and hyper polarizabilities were calculated.

EXPERIMENTAL

Reagents and apparatus

All chemical substances of analytical reagent grade were supplied by E. Merck AG (Darmstadt, Germany) and accepted for use without further purification. Stock solutions were prepared by dissolving the appropriate amount of ceftriaxone in 200 mM phosphate buffer solution (PBS) at pH 7.0 (which was also used as the supporting electrolyte) by directly dissolving it in twice distilled water. The working solutions of phenylalanine and ceftriaxone were obtained by diluting their stock solutions with PBS. All reagents used freshly prepared. The pH measurements were made using a Metrohm 632 Digital pH-meter (Metrohm AG, CH-9100 Herisau, Switzerland) with a combined glass electrode. Electrochemical measurements were carried out with PC-controlled BASi potentiostat C3 stand connected with a N_2 gas cylinder, produced by Bioanalytical System Inc., (BASi) USA. The working electrode was a glassy carbon electrode (GCE) (2 mm diameter). Before use the working electrode was sequentially polished with graded 10 μM alumina powder, and rinsed with doubly distilled water. A saturated Ag/AgCl and a platinum wire were used as the reference and the auxiliary electrode, respectively. This three-electrode micro-cell was completely shielded from any perturbing noises by a Faraday Cage. A continuous flow of nitrogen was ensured before start of any electrochemical experiment. All solutions were purged with pure nitrogen for 10 min before the voltammetric runs. Cyclic voltammetry (CV) measurements were made at a 50 mV s^{-1} scan rate.

Calculation methods

All calculations were performed with complete geometry optimization by using the standard Gaussian 09 software package with DFT and B3LYP hybrid method with 6-311G(d) basis sets [17]. Fukui function indicating the change in electron density of a molecule at a given position, when the number of electrons has changed, were calculated by using AOMix program from singlepoint calculations with B3LYP/6-311G(d,p) [18-19].

RESULTS AND DISCUSSION

Influence of scan rate

Cyclic voltammetry (CV) of ceftriaxone and ceftriaxone phenylalanine (PA) systems were carried out separately. We examined the influence of the scan rate on the electrochemical behavior of ceftriaxone, to understand the nature of the electrode process. For this, we recorded the cyclic voltammogram of the 5×10^{-4} M ceftriaxone on GCE in 0.2 M phosphate buffer solution at pH 7.0. The cathodic peak currents increased linearly when the scan rate varied from 25 to 275 mVs^{-1} and the correlation coefficient was found to be 0.9719 and 0.9592 for ceftriaxone and ceftriaxone-PA systems, respectively, in the potential range of 0.8-(-0.2) V. The reduction peak current of ceftriaxone was noted to increase with increasing scan rate.

On addition of PA, a decrease in the cathodic peak current was observed. The competitive adsorption between ceftriaxone and PA on the GCE for the formation of electroinactive complex without the changes of electrochemical parameters may be effective for decreasing of the reductive peak current. The peak current of ceftriaxone did not disappear completely with the increase in the concentration of PA, which was not typical for competitive adsorption. The competitive adsorption factor can be excluded by recording a cyclic voltammogram of ceftriaxone in the excess of PA.

Consequently, the decrease in the peak current without any changes in electrochemical parameters is an evidence of [ceftriaxone-PA] electroinactive

complex formation, which results in the decrease of equilibrium concentration of ceftriaxone in solution.

The effect of scan rate on peak current showed that the plot of current *vs.* υ is linear for a limited scan rate, indicating that the electrochemical process is virtually an adsorption-controlled process. However, the process is also diffusion-controlled, as shown by the *I* peak *versus* υ1/2 plot in Fig. 1. It can be concluded that the electrochemical process of the ceftriaxone-PA system actually includes both diffusion and adsorption-controlled processes, depending on the scan rate.

Voltammetric study of ceftriaxone, ceftriaxone and phenylalanine systems

The probable interaction of ceftriaxone and phenylalanine was studied by comparing the voltammetric data between ceftriaxoneand, ceftriaxone – phenylalanine systems in aqueous PBS solution.

The voltammetric behavior of 5×10^{-4} M ceftriaxone in the absence and presence of PA at bare GCE is shown in Figs. 2 and 3, respectively. The voltammogram without PA featured a cathodic peak in the potential range of (-0.2) – 0.8 V. As it is shown, by increasing the scan rate, the peak potential is shifted to lower positive potentials.

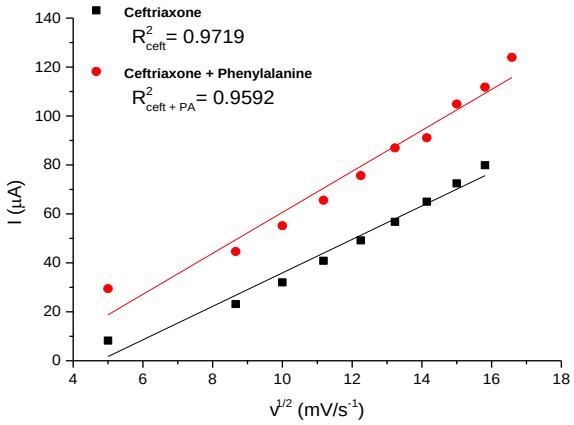

Figure 1. Plot of I peak *vs.* $\upsilon^{1/2}$ for 5×10^{-4} M ceftriaxone in absence and presence of PA (5×10^{-4} M) at various scan rates: 25,50,75,100125,150,175,200,225,250 and 275 mV s^{-1}

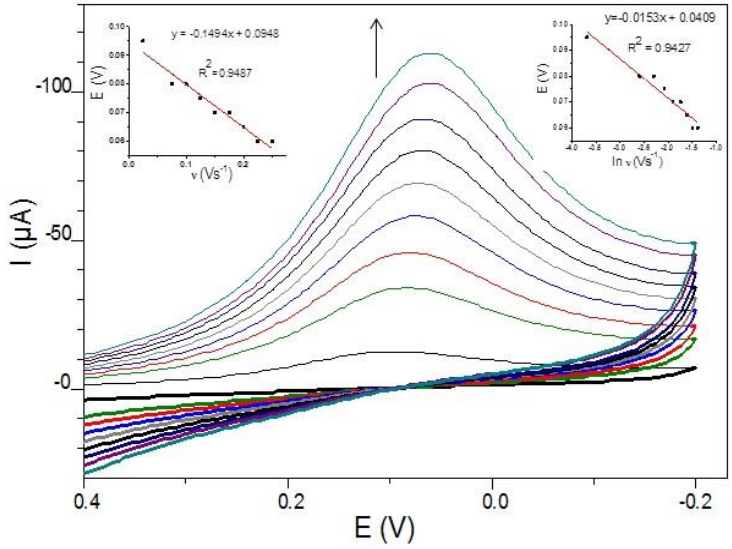

Figure 2. Cyclic voltammograms of 0.5 mM ceftriaxone at various scan rates. Inset left: linear plot of Ep *vs.* υ (y=−0.1494x+0.0948, R^2=0.948). Inset right: linear plot of Ep *vs.* ln (υ/V s^{-1}) (y=−0.0153x+0.0409, R^2=0.943). pH 7.0 PBF solution at glassy carbon electrode. Scan rates: 25,75,100,125,150,175,200,225 and 250 mV s^{-1}.

The electron transfer rate constant (*k*s) and αn were calculated using Laviron's equations for the irreversible surface electrode process of the reduction of ceftriaxone [20-21]:

$$E = E^0 + \left(\frac{RT}{\alpha F}\right)\ln\left[\frac{RTk_s}{\alpha F}\right] - \left[\frac{RT}{\alpha F}\right]\ln[\upsilon] \quad (1)$$

where R is the universal gas constant R = 8.314 J mol^{-1} K^{-1}, T is Kelvin temperature T = 298 K, F is the Faraday constant F = 96487 C mol^{-1} ,α is the electron transfer coefficient, k_s is the standard rate constant of the surface reaction. The results are shown in Table 1. υ is the scan rate (Vs^{-1}) and E$^{0'}$ is the formal potential. If the E$^{0'}$ is known, k_s and α values can be calculated according to the linear plot of *E versus* ln υ .(insets right, (Figs. 2 and 3)). The E$^{0'}$ value can be obtained from the intercept of E vs. υ plot .(insets left, (Figs. 2 and 3)).

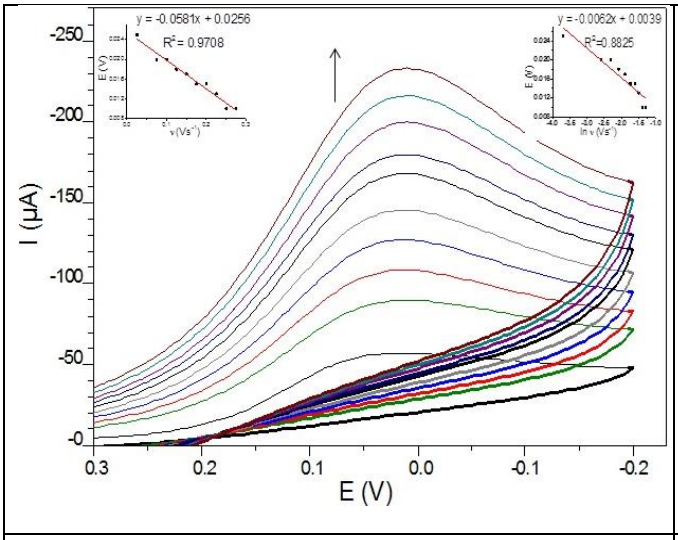

Figure 3. Cyclic voltammograms of 0.5 mM ceftriaxone + 0.5 mM PA at various scan rates. Inset left: linear plot of Ep *vs.* υ (y=−0.0581x+0.0256, R^2=0.971). Inset right: linear plot of Ep *vs.* ln (υ/V s^{-1}) (y=−0.0062x+0.0039, R^2=0.883). pH 7.0 PBF solution at glassy carbon electrode. Scan rates: 25,75,100,125,150,175,200,225,250 and 275 mV s^{-1}

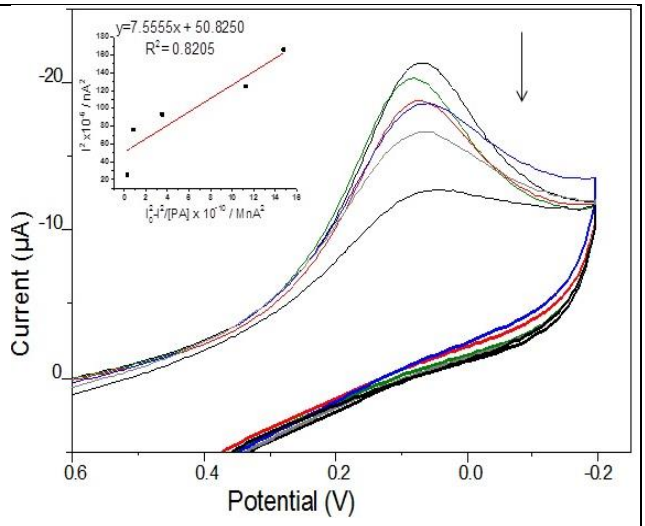

Figure 4. Cyclic voltograms of 0.5 mM ceftriaxone in 0.2 M phosphate buffer of pH 7.0 without PA and in the presence of C_{PA}= 0.1,0.5,2.5,12.5,62.5 mM PA at 50 mV s^{-1}. Inset left: plot of I^2 *vs.* (I_0^2- I^2)/[PA] used to calculate the binding constant

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Table 1. E^0, k_s and α values of ceftriaxone in the absence and presence of PA.

	Ceftriaxone	Ceftriaxone + PA
E^0 *(V)*	0.094	0.026
α	1.678	4.142
k_s *(s^{-1})*	2.031	4.831

Cyclic voltammetric measurements of PA with ceftriaxone were carried out to determine the binding constant using the following equation:

$$I^2 = \frac{1}{K[PA]}\,(I_0^2 - I^2) + I_0^2 - [PA] \qquad (2)$$

where I_0 and I are the peak currents of [ceftriaxone] in the absence and presence of PA, respectively [22]. A plot of I^2 *vs.* (I_0^2 – I^2)/[PA] was described with a straight line to give a binding constant of $K = 1.32 \times 10^3\,M^{-1}$ (Fig. 4).

Theoretical aspects

The reaction between ceftriaxone and phenyl alanine was thought as two forms. One of the reactions was performed between the carboxyl group of ceftriaxone and the amino group of phenylalanine (compund A) the other one was considered between the carboxyl group of phenylalanine and the amino group of ceftriaxone (compound B).

Optimized form, the highest occupied molecular orbital - HOMO, and the lowest molecular orbital – LUMO, and electron density of ceftriaone, molecule A, molecule B, calculated with B3LYP/ 6-311++g(2d,2p) are given in Fig. 5. There are three rings in ceftriaxone. These are triazine (ring 1), azobicyclo (ring 2) and thiazole (ring 3). The calculation of ceftriaxone molecule was performed in cationic form. HOMO of the cationic form of ceftriaone mainly consists of ring 1, ring 3, carbonyl and amino groups. LUMO of the cationic form of ceftriaone mainly consists of ring 1, ring 2, and ring 3. HOMO of the complex A is mainly concentrated on ring 3 and the imino group attached to ring 3. LUMO of the complex A mainly consists of ring 3. HOMO of the complex B is mainly concentrated on ring 1 and LUMO of the complex A mainly consists of ring 3. The common descriptors of site reactivity are related wirh Fukui functions which can be enunciative in a finite-difference approximation by the following equations:

$$f_k^+ = \rho_k(N+1) - \rho_k(N) \qquad (3)$$

$$f_k^- = \rho_k(N) - \rho_k(N-1) \qquad (4)$$

The first equation (3) expresses a condensed Fukui function for a nucleophilic attack; the second equation (4) means a condensed Fukui function for an electrophilic attack. Table 2 shows the compositions of the HOMO and the LUMO of the molecule calculated at B3LYP/6-311G(d,p) level.

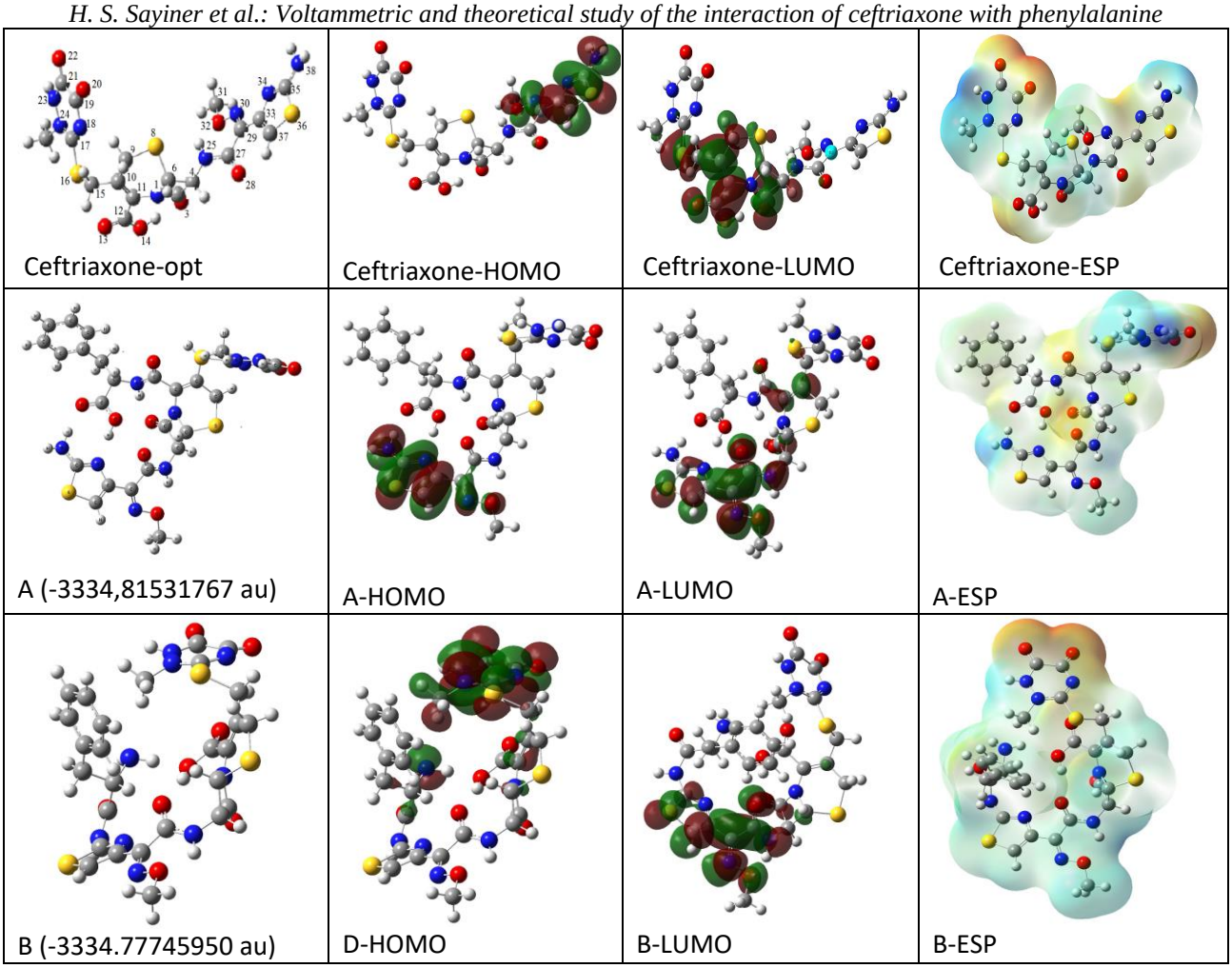

Figure 5. Optimized form, HOMO, LUMO, and electron density of ceftriaxone, molecule A, molecule B

Table 2. Compositions of the HOMO and the LUMO of the caftriaxone, A and B molecule at the B3LYP/6-311G(d,p) level

Ceftriaxone			A			B		
Atom No	HOMO	LUMO	Atom No	HOMO	LUMO	Atom No	HOMO	LUMO
C2		9.89	C10		2.55	S16	3.86	
O3		5.33	N25		3.62	N18	12.29	
C10		29.61	C26		11.36	O20	7.66	
C11		17.27	O27		8.67	O22	12.95	
C12		8.66	O28		21.29	N23	20.97	
O13		2.64	C29	4.89	26.69	N24	25.08	
O14		6.82	O30	3.05	3.54	N26		4.30
C15		3.07	C32	13.61	2.15	C27		11.76
S16		5.97	C33	9.00		O28		7.76
N30	7.76		N34	7.32		29C		20.05
O31	4.97		C35	11.87	2.83	N30		27.33
C33	14.76		S36	24.66	7.59	31O		3.96
N34	5.66		C37	23.24		33C		2.62
C35	9.28					S36		4.74
S36	11.43					C37		11.35
C 37	25.27					N53	4.32	
38N	17.76							

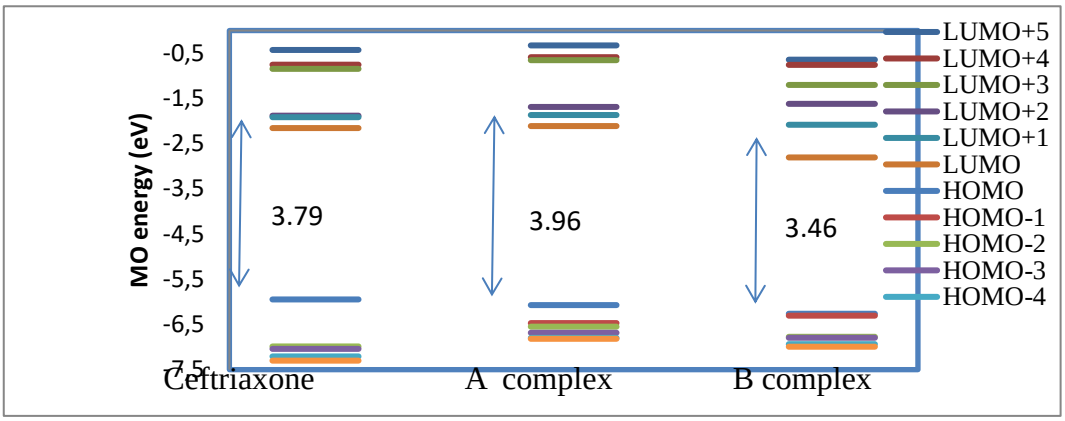

Figure 6. E_{HOMO}, E_{LUMO}, and the energy of 5 molecular orbitals close to these orbitals ceftriaxone, A and B molecules

The HOMO composition represents the condensed Fukui function for an electrophilic attack. For the HOMO, the contributions of the C33, N34, C35, S36, C37 for thiazole group of ceftriaxone are 14.76 %, 5.66 %, 9.28 %. 11.43 %, 25.27 %, respectively. For the HOMO, the contributions of C33, C34, C35, S36, C37 belonging to the thiazole group of complex A are 13.61 %, 9.00 %, 7.32 %, 11.87 %. 24.66 %, 23.24 %, respectively. The contributions of N18, O22, N23, N24 of the triazine group of complex B are 12.29 %, 12.95 %, 20.97 %, 25.08 %, respectively.

The LUMO composition represents the condensed Fukui function for a nucleophilic attack. The LUMO contributions mainly belong to azobicyclo group and thiazole group of ceftriaxone.

CONCLUSION

The electrochemical investigation of the redox behavior of ceftriaxone and the interaction of ceftriaxone with phenyl alanine was performed. From our findings we conclude that ceftriaxone reacts wih phenylalanine following an irreversible charge transfer reaction at a glassy carbon electrode. Cathodic currents of ceftriaxone-phenyl alanine were reduced. This is the evidence of [ceftriaxone-PA] electroinactive complex formation. According to the theoretical calculation form A is preferred to form B.

For complex A the compositions belong to S35, C36 on the thiazole group and the groups attached to thiazole group (for N25, C26, O27, C28, C29, O30 are 3.62 %, 11.36 %, 8.67 %, 21.29 %, 26.69%, 3.54%, respectively). For complex B the compositions belong to C33, S36, C37 (2.62 %, 4.74 %, 11.35 %) on the thiazole group and the groups attached to the thiazole group (for N26, C27, O28, C29, N30, O31 are 4.30 %, 11.76 %, 7.76 %, 20.05 %, 27.33%, 3.96%, respectively).

The highest occupied molecular orbital energy (E_{HOMO}), the lowest unoccupied molecular orbital energy (E_{LUMO}), and the energy of 5 molecular orbitals close to these orbitals energetically are given in Fig. 6. Energy gaps for ceftriaxone, A and B molecules are 3.79, 3.96, 3.46 eV, respectively.

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ВОЛТАМПЕРОМЕТРИЧНО И ТЕОРЕТИЧНО ИЗСЛЕДВАНЕ НА ВЗАИМОДЕЙСТВИЕТО МЕЖДУ ЦЕФТРИАКСОН И ФЕНИЛАЛАНИН

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(Резюме)

Взаимодействието между цефтриаксон и фенилаланин (PA) е изследвано чрез циклична волтамперометрия и квантово-химични изчисления с помощта на DFT метод. Изследването е проведено във фосфатен буфер с pH 7 (използван също като поддържащ електролит чрез директно разтваряне в двойно дестилирана вода). Волтамперометричното изследване на цефтриаксон показа добре изразени редокс пикове при 0.090 V върху електрод от стъкловъглерод във фосфатен буфер с pH 7 при 50 mVs^{-1}. Катодните пикови токове са линейни при скорост на сканиране от 25 до 275 mVs^{-1} и корелационният коефициент е съответно 0.9719 и 0.9592 за цефтриаксон и цефтриаксон-PA системите в интервала от потенциали 0.8-(-0.2) V. Скоростната константа на електронен пренос (k_s) е изчислена за редукцията на цифтриаксон и взаимодействието между цефтриаксон-PA, съответно 2.031 и 4.831 s^{-}. След добавяне на PA към разтвора на цефтриаксон е получена константата на редокс свързване K = 1.32×10^3 M^{-1} за взаимодействието цефтриаксон-PA. Квантово-химични изчисления за цефтриаксон и цефтриаксон-PA комплекс са проведени с помощта на B3LYP метод.