Cephalexin degradation initiated by OH radicals: theoretical prediction of the mechanisms and the toxicity of byproducts

R. Masmoudi1 · S. Khettaf1 · A. Soltani1 · A. Dibi1 · L. Messaadia2 · M. Benamira3

Received: 20 December 2021 / Accepted: 18 April 2022 / Published online: 10 May 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
In this work, the density functional theory is used to study the local reactivity of cephalexin (CLX) to radical attack and explain the mechanism of the reaction between CLX and hydroxyl radical attack leading to degradation byproducts. The reaction between •OH and CLX is supposed to lead to either an addition of a hydroxyl radical or an abstraction of a hydrogen. The results showed that the affinity of cephalexin for addition reactions increases as it passes from the gas to the aqueous phase and decreases as it passes from the neutral to the ionized form. Thermodynamic data confirmed that OH addition radicals (Radd) are thermodynamically favored over H abstraction radicals (Rabs). The ecotoxicity assessments of CLX and its byproducts are estimated from the acute toxicities toward green algae, Daphnia, and fish. The formation of byproducts is safe for aquatic organisms, and only one byproduct is harmful to Daphnia.

Keywords Quantum chemical calculations · DFT · Advanced oxidation processes · Cephalexin · Hydroxyl radical · Wastewater treatment

Introduction
Growing concentrations of antibiotics and their metabolites in aqueous environments have caused great concerns to human health and different ecological systems. Cephalexin, (6R,7R)-7-[(2R)-2-amino-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1 azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (CLX), is a first-generation cephalosporin used in human and veterinary medicine in the treatment of bacterial infections [1]. Cephalexin is frequently detected in the effluents of wastewater treatment plants (WWTPs) and exhibits very low biodegradability [3]. Consequently, the World Health Organization (WHO) warned about the presence of pharmaceuticals in drinking water and the potential implications on human health and the environment and classified cephalosporin antibiotics as emergent environmental contaminants [4]. WWTPs use advanced oxidation processes (AOPs) to remove contaminants from wastewater. This technique is based on the in situ generation of reactive oxygen species (ROS) and hydroxyl radicals (•OH) through chemical processes that employ ozone and hydrogen peroxide combined with irradiation of UV light, electromagnetic radiation, and ultrasound [5–7]. The advantage of •OH is that it is highly reactive and nonselective with a large range of organic compounds [8]. There are numerous methods for the determination of local reactivity descriptors. The study of He et al. [2] located the reactive sites of cephalexin on the basis of frontier electron densities $\text{FED}_\text{HOMO}$ and $\text{FED}_\text{LUMO}$ for each site. The research established by Cao et al. [9] compared the different methods used for local reactivity determination (nucleophilic, electrophilic, and radical reactive sites), from which Fukui function proved to be one of most efficient methods for predicting local reactivity.
The most accurate approach is the one proposed by Yang and Mortier [10] using the atom condensed Fukui function. The byproduct structures of CLX hydroxyl-mediated degradation have been reported by some authors [11–15], while the thermodynamic data and a mechanism of the degradation process are still uncovered. Previous studies indicate that the most common degradation pathways include hydroxylation, decarboxylation, demethylation, and dealkylation [2, 12, 13]. Another study claimed that the majority of oxidation intermediates result from OH attack on the CLX cephalosporin ring, where the intermediates undergo opening of the active site of the molecule responsible for the antibacterial activity, and another intermediate formation pathway involves the electrophilic addition of OH to the aromatic moiety [16].

Terminal degradation products such as lactic, acetic, and propionic acids are rapidly further oxidized (mineralization processes). However, fumaric, oxalic, and oxamic acids are hard to further oxidize. More recently, there has been a growing interest in predicting the photodegradation mechanism, kinetics, and ecotoxicological assessment of water-emerging contaminants (EOCs) with the assistance of theoretical calculations. Several experimental studies have been conducted on cephalexin hydroxyl–mediated degradation, e.g., sonochemical processes [17], electrochemical advanced oxidation processes [16], photo-Fenton processes [18], and photocatalytic nanowire oxidation processes [2], but little is known about its degradation mechanism. This work is dedicated to assess the safety of advanced oxidation processes (AOPs) and as a theoretical back-up of the previous experimental works on the degradation of cephalexin. The density functional theory is used to study the local reactivity of cephalexin (CLX) to radical attack and explain the mechanism of the reaction between CLX and hydroxyl radical attack leading to degradation byproducts. Moreover, the ecotoxicity assessments of CLX and its byproducts are estimated.

Materials and methods

Quantum chemical calculations

All calculations were performed with the Gaussian 16 program package. Geometry optimization was conducted using the DFT/B3LYP/6–31 + G(d) basis set [19]. Stationary points were characterized by frequency analysis where the free energy and thermal enthalpy (kcal/mol) were obtained at 298 K. Conductor-like Polarizable Continuum Model (CPCM), one of the most efficient models applied to compute the effect of solvent in quantum chemical calculations, was used to study the effect of the solvent environment, i.e., water ($\epsilon = 78.36$) [20]. The pKa (unitless constant which shows how strong or weak an acid is) of cephalexin was calculated in this work using the ChemAxon Marvin Sketch software, 2.56 for the carboxylic function and 6.88 for the primary amine function [21]. Since degradation takes place in a medium at pH 7 (for water), the degradation of CLX was modeled into neutral and anionic forms in both gas and aqueous phases.

Fukui indices were an important idea that has been widely used to predict the reactive sites of electrophilic, nucleophilic, and radical attacks. The sites with higher values of Fukui indices on molecule structure were considered preferentially reactive. The Fukui indices ($f_k^+, f_k^-$, and $f_k^0$) were calculated from the values of the population of Mulliken or NBO (natural bond orbital) analysis by the following formulas [22]:

\[
f_k^+ = q_k(N + 1) - q_k(N) \quad \text{for nucleophilic attack} \tag{1}
\]

\[
f_k^- = q_k(N) - q_k(N - 1) \quad \text{for electrophilic attack} \tag{2}
\]

\[
f_k^0 = \frac{1}{2} [q_k(N + 1) - q_k(N - 1)] \quad \text{for radical attack} \tag{3}
\]

where $q_k(N)$ is the atomic charge (Mulliken charge) on the $k$ atomic site of the molecule with $N$ electrons in neutral ($N$), cationic ($N - 1$), and anionic ($N + 1$) chemical species. $q_k(N + 1)$ and $q_k(N - 1)$ are the charges on the $k$-atom of the molecule with $(N - 1)$ and $(N + 1)$ electrons in a frozen orbital approximation, respectively.

Equation (5) and Eq. (7) were used to calculate the stability of the generated radicals of OH addition and H abstraction, respectively [23, 24]:

\[
\Delta G_{rxn} = G(R_{add}) - G(\cdot OH) - G(CLX) \tag{5}
\]

\[
\Delta G_{rxn} = G(H_2O) + G(R_{abs} \cdot +H_2O) - G(\cdot OH) - G(CLX) \tag{7}
\]

In addition to the above measures, the standard radical stabilization energies ($\text{RSE} = \Delta H^0_{298}$) of the produced radicals were calculated at 298 K using Eq. (9), where $R \cdot$ is the concerned radical.

\[
\Delta H^0_{298} = \Delta H_{298}^0(CH_3 \cdot) + \Delta H_{298}^0(R - H) - \Delta H_{298}^0(CH_4) - \Delta H_{298}^0(R \cdot) \tag{9}
\]

$\Delta H$ is the enthalpy change of the reaction (kcal/mol). In the case where it corresponds to a positive value, $R \cdot$ is expected to be more stable than $CH_3 \cdot$; otherwise, $R \cdot$ is expected to be less stable than $CH_3 \cdot$.
The Gibbs free energies of fragmentation (kcal/mol) help to identify the most suitable fragmentation pathways of CLX/CLX⁻, which are calculated using Eq. (10):

\[
\Delta G_{\text{rxn}} = (G(\text{fragment 1}) + G(\text{fragment 2})) - G(\text{CLX})
\]  

(10)

Fig. 1 a Species distribution diagram of cephalexin as a function of solution pH. b Molar fractions (%) of the main different species of cephalexin.
Ecotoxicity assessment

The ecotoxicological impact of cephalexin and its degradation byproducts were estimated for three tropical levels (fish, green algae, and Daphnia) in terms of both efficient and lethal concentrations. Ecological Structure Activity Relationship (ECOSAR) software was used in this study [25]. ECOSAR Class Program is a computerized predictive system used to predict the aquatic toxicity. The program estimates a chemical’s acute toxicity and chronic toxicity to aquatic organisms, such as fish, aquatic invertebrates, and aquatic plants, by using computerized Structure Activity Relationships (SARs). EC_{50} refers to the pollutant level of the tested compound that could inhibit 50% growth of green algae after 96 h of exposure time. LC_{50} represents the concentration of the tested pollutant that causes the death of 50% of fish and Daphnia after 96 and 48 h of contact times, respectively.

Results and discussion

Initial reaction of CLX with •OH

In the aqueous phase, cephalexin can exist in six forms depending on the pH of the solution (Fig. 1). The ChemAxon
Table 1: Fukui indices of neutral cephalixin in gas phase using DFT/B3LYP/6–31+G(d)

| Atoms | $q_k(N)$ | $q_k(N-1)$ | $q_k(N+1)$ | $f_k^+$ | $f_k^-$ | $f_k^0$ |
|-------|----------|------------|------------|--------|--------|--------|
| C6    | -0.16    | 0.26       | 0.14       | -0.31  | -0.09  | -0.2   |
| H7    | 0.20     | 0.21       | 0.21       | 0      | 0      | 0      |
| H8    | 0.20     | 0.21       | 0.17       | -0.01  | -0.02  | 0.01   |
| H9    | 0.19     | 0.21       | 0.16       | -0.01  | -0.03  | 0.02   |
| H10   | 0.20     | 0.21       | 0.16       | -0.01  | -0.03  | 0.02   |
| H11   | 0.19     | 0.21       | 0.17       | -0.01  | -0.02  | 0.01   |
| H13   | 0.21     | 0.24       | 0.19       | -0.02  | -0.02  | 0.02   |
| C14   | -0.10    | -0.08      | -0.08      | -0.01  | 0.01   | 0      |
| H17   | 0.48     | 0.47       | 0.47       | 0      | -0.01  | 0      |
| H20   | 0.27     | 0.3        | 0.24       | -0.02  | -0.03  | 0.03   |
| H21   | 0.27     | 0.28       | 0.22       | -0.01  | -0.04  | 0.02   |
| H23   | 0.27     | 0.29       | 0.2        | -0.01  | -0.06  | 0.04   |
| H24   | 0.29     | 0.3        | 0.22       | -0.01  | -0.06  | 0.04   |
| C25   | 1.93     | 1.46       | 1.78       | 0.46   | -0.14  | -0.16  |
| C26   | -1.21    | -0.71      | -1.52      | -0.49  | -0.31  | 0.40   |
| H28   | 0.24     | 0.25       | 0.23       | -0.01  | 0      | 0.01   |
| H29   | 0.23     | 0.24       | 0.19       | -0.01  | -0.04  | 0.02   |
| H30   | 0.24     | 0.27       | 0.16       | -0.02  | -0.08  | 0.05   |
| C31   | 0.19     | 0.15       | 0.84       | 0.04   | 0.64   | -0.34  |
| H34   | 0.53     | 0.53       | 0.48       | 0      | -0.04  | 0.02   |
| H38   | 0.42     | 0.42       | 0.4        | 0      | -0.01  | 0      |
| H39   | 0.41     | 0.40       | 0.38       | 0      | -0.02  | 0.01   |
| C40   | 0.88     | 0.86       | 0.57       | 0.01   | -0.3   | 0.14   |

Table 2: Fukui indices of neutral cephalixin in aqueous phase using DFT/B3LYP/6–31+G(d)

| Atoms | $q_k(N)$ | $q_k(N-1)$ | $q_k(N+1)$ | $f_k^+$ | $f_k^-$ | $f_k^0$ |
|-------|----------|------------|------------|--------|--------|--------|
| C6    | -0.21    | 0.22       | 0.19       | -0.01  | 0      | 0      |
| H7    | 0.20     | 0.21       | 0.20       | -0.01  | 0.01   | 0      |
| H8    | 0.18     | 0.20       | 0.2        | -0.02  | 0.01   | 0      |
| H9    | 0.18     | 0.20       | 0.19       | -0.02  | 0.01   | 0      |
| H10   | 0.18     | 0.20       | 0.20       | -0.01  | 0      | 0      |
| H11   | 0.18     | 0.19       | 0.19       | 0.01   | 0.02   | -0.02  |
| H13   | 0.20     | 0.22       | 0.21       | -0.01  | 0.04   | -0.01  |
| C14   | -0.13    | -0.12      | -0.09      | 0.07   | -0.12  | 0.02   |
| H17   | 0.47     | 0.49       | 0.41       | -0.03  | 0.14   | -0.05  |
| H20   | 0.27     | 0.31       | 0.26       | -0.06  | 0      | -0.03  |
| H21   | 0.24     | 0.31       | 0.25       | 0.12   | 0.22   | -0.17  |
| H23   | 0.25     | 0.23       | 0.31       | -0.07  | -0.02  | 0.04   |
| H24   | 0.26     | 0.24       | 0.33       | 0.17   | -0.11  | 0.03   |
| C25   | 1.77     | 1.59       | 1.66       | -0.56  | -0.39  | 0.48   |
| C26   | -1.11    | 0.55       | 1.51       | -0.01  | -0.29  | 0.15   |
| H28   | 0.23     | 0.26       | 0.22       | -0.03  | -0.03  | 0.03   |
| H29   | 0.23     | 0.27       | 0.20       | -0.08  | -0.02  | 0.05   |
| H30   | 0.21     | 0.29       | 0.19       | 0.12   | 0.53   | -0.33  |
| C31   | 0.21     | 0.08       | 0.74       | -0.01  | -0.3   | 0.15   |
| H34   | 0.50     | 0.51       | 0.55       | -0.07  | -0.23  | 0.15   |
| H38   | 0.39     | 0.42       | 0.43       | -0.01  | 0      | 0      |
| H39   | 0.40     | 0.41       | 0.41       | 0.05   | -0.16  | 0.05   |
| C40   | 0.87     | 0.71       | 0.82       | -0.07  | -0.15  | 0.11   |
Marvin Sketch software was used to calculate the pKa and to predict which structure of acid or base will dominate in the

| Atoms | \( q_k(N) \) | \( q_k(N-1) \) | \( q_k(N+1) \) | \( f_k^+ \) | \( f_k^- \) | \( f_k^0 \) |
|-------|-----------|-----------|-----------|----------|----------|----------|
| C6    | 0.26      | -0.17     | -2.50     | -2.77    | 0.44     | -11.61   |
| H7    | 0.21      | 0.19      | 0.18      | -0.02    | 0.01     | 0        |
| H8    | 0.18      | 0.18      | 0.14      | -0.04    | 0        | -0.02    |
| H9    | 0.16      | 0.18      | 0.13      | -0.03    | -0.01    | -0.02    |
| H10   | 0.17      | 0.18      | 0.14      | -0.02    | -0.01    | -0.01    |
| H11   | 0.17      | 0.18      | 0.19      | 0.01     | 0        | 0        |
| H13   | 0.19      | 0.2       | 0.2       | 0.01     | -0.01    | 0        |
| C14   | -0.13     | -0.17     | -3.93     | -3.79    | 0.04     | -1.87    |
| H17   | 0.47      | 0.47      | 0.48      | 0.01     | 0        | 0        |
| H20   | 0.25      | 0.28      | 0.25      | 0        | -0.03    | -0.01    |
| H21   | 0.23      | 0.26      | 0.22      | -0.01    | -0.02    | -0.01    |
| H23   | 0.22      | 0.26      | 0.21      | -0.01    | -0.03    | -0.02    |
| H24   | 0.24      | 0.27      | 0.23      | -0.01    | -0.03    | -0.02    |
| C25   | 1.44      | 1.25      | 1.31      | -0.13    | 0.18     | 0.02     |
| C26   | -1.09     | -0.35     | -0.96     | 0.13     | -0.74    | -0.3     |
| H28   | 0.27      | 0.26      | 0.26      | -0.01    | 0.01     | 0        |
| C31   | 0.38      | -0.28     | 0.36      | -0.02    | 0.66     | 0.32     |
| H37   | 0.4       | 0.4       | 0.38      | 0        | -0.01    | 0        |
| H38   | 0.39      | 0.4       | 0.38      | 0        | -0.01    | 0        |
| C39   | 0.67      | 0.95      | 0.7       | 0.03     | -0.28    | -0.12    |
| C40   | -0.48     | -0.41     | -0.5      | -0.02    | -0.06    | -0.04    |

Table 3  Fukui indices of ionized cephalexin in gas phase using DFT/B3LYP/6–31 + G(d)

| Atoms | \( q_k(N) \) | \( q_k(N-1) \) | \( q_k(N+1) \) | \( f_k^+ \) | \( f_k^- \) | \( f_k^0 \) |
|-------|-----------|-----------|-----------|----------|----------|----------|
| C6    | -0.22     | -0.18     | 0         | 0        | -0.03    | -0.02    |
| H7    | 0.21      | 0.21      | 0.2       | 0        | 0        | 0        |
| H8    | 0.2       | 0.2       | 0.2       | 0        | 0        | 0        |
| H9    | 0.19      | 0.2       | 0.19      | 0        | 0        | 0        |
| H10   | 0.2       | 0.2       | 0.2       | 0        | 0        | 0        |
| H11   | 0.19      | 0.19      | 0.19      | 0        | 0        | 0        |
| H13   | 0.21      | 0.22      | 0.2       | 0        | 0        | 0        |
| C14   | -0.08     | -0.11     | 0         | 0        | 0.02     | 0        |
| H17   | 0.48      | 0.49      | 0.46      | -0.02    | 0        | -0.01    |
| H20   | 0.27      | 0.29      | 0.2       | -0.06    | -0.02    | -0.04    |
| H21   | 0.26      | 0.3       | 0.24      | -0.02    | -0.03    | -0.03    |
| H23   | 0.26      | 0.29      | 0.23      | -0.02    | -0.03    | -0.03    |
| H24   | 0.27      | 0.31      | 0.25      | -0.02    | -0.04    | -0.03    |
| C25   | 1.62      | 1.39      | 1.85      | 0.23     | 0.22     | 0.23     |
| C26   | -1.05     | -0.36     | -0.94     | 0.10     | -0.68    | -0.29    |
| H28   | 0.23      | 0.25      | 0.21      | -0.01    | -0.02    | -0.02    |
| H29   | 0.22      | 0.26      | 0.2       | -0.01    | -0.04    | -0.02    |
| H30   | 0.22      | 0.27      | 0.2       | -0.01    | -0.04    | -0.03    |
| C31   | 0.12      | 0.22      | -0.05     | -0.17    | -0.1     | -0.13    |
| H37   | 0.43      | 0.43      | 0.42      | 0        | 0        | 0        |
| H38   | 0.41      | 0.41      | 0.41      | 0        | 0        | 0        |
| C39   | 0.87      | 0.91      | -0.76     | -1.64    | -0.04    | -0.84    |

Table 4  Fukui indices of ionized cephalexin in aqueous phase using DFT/B3LYP/6–31 + G(d)
pH range of 0–14. In this diagram of the species repartition presented in terms of molar fractions (%), six forms were observed. In the first region (pH range of 0–4), the predominant species is C, with molar fractions of 99.91%. The first deprotonation takes place at the OH of carboxylic acid, with a relative pKa value of 3.26 (Fig. 2). The second region is described by the B species from pH 7 to 12 with molar fractions of 99.09%. In this case, deprotonation took place at the primary amine NH₂ functional group (pKa = 7.23), and these results are in good agreement with the literature [26, 27]. In the third region, from pH = 4 to 7, the most important species is D in a zwitterion form with a molar fraction of 97.95%. The other species (E, F, and G) existed in the pH region of 10–14 with molar fractions of 53.43, 12.40, and 94.97%, respectively.

Fig. 4 The sites concerned with radical attack on neutral cepalexin in the gas (a) and aqueous phase (b)

Fig. 5 The sites concerned with radical attack on ionized cepalexin in the gas (a) and aqueous phase (b)

Fig. 6 H abstraction by •OH from CLX, generating radical intermediate (Rabs₁)
Cephalexin has two functions mainly responsible for the acid base behavior in the molecule, a carboxylic acid function and primary amine, and the degradation of cephalexin takes place in a medium at pH = 7 (for water). This allows us to model the molecule in both neutral and anionic forms. The solvent intervenes in the geometric parameters by influencing, mainly, the bonds of the aliphatic chain and, by a less remarkable effect, the bonds of the cephem ring, while the lengths of the bonds of benzene are not practically affected. The most stable conformers of the neutral and anionic forms are depicted in Fig. 3.

Advanced oxidation processes (AOPs) produce powerful nonselective oxidant agents, mainly •OH radicals, which interact with contaminant molecules to initiate the degradation process [7]. Information on the behavior of cephalexin in the presence of •OH radicals is first required to determine the most likely sites in view of radical attack. The reactivity of cephalexin toward •OH is supposed to be either by hydrogen abstraction or by hydroxyl addition. The concerned sites on cephalexin molecules are either hydrogen atoms, in which the outcome of their interaction with hydroxyl radicals is an abstraction reaction, or unsaturated carbon atoms, in which the result of their interaction with hydroxyl radicals is an addition reaction [28]. The reactivity of the candidate sites is studied through atom condensed Fukui index analysis. These indices indicate the susceptibility of a site to gain or lose an

\[
\begin{align*}
\text{Table 5} & \quad \text{Free energies, enthalpies, and RSE of abstraction radicals in gas phase using DFT/B3LYP/6–31 + G(d)} \\
& \quad \text{(all values are in kcal/mol)} \\
& \quad \begin{array}{cccc}
R_{\text{abs}}(–H38) & -0.94 & -17.25 & -0.08 \\
R_{\text{abs}}(–H34) & -0.66 & -16.69 & -0.36 \\
R_{\text{abs}}(–H13) & -0.004 & -37.58 & -1.02 \\
R_{\text{abs}}(H28/29/30) & 0.08 & -30.62 & -1.11 \\
R_{\text{abs}}(–H9) & 0.09 & -2.07 & -1.12 \\
R_{\text{abs}}(–H8/H10) & 0.09 & -2.32 & -1.12 \\
R_{\text{abs}}(–H11) & 0.13 & -2.32 & -1.16 \\
R_{\text{abs}}(H20) & 0.14 & -27.73 & -1.16 \\
R_{\text{abs}}(–H21) & 0.27 & -21.27 & -1.30 \\
R_{\text{abs}}(–H23/H24) & 0.28 & -40.09 & -1.31 \\
\end{array}
\end{align*}
\]

\[
\begin{align*}
\text{Table 6} & \quad \text{Free energies, enthalpies, and RSE of abstraction radicals in aqueous phase radicals using DFT/B3LYP/6–31 + G(d)} \\
& \quad \text{(all values are in kcal/mol)} \\
& \quad \begin{array}{cccc}
R_{\text{abs}}(–H38) & -0.94 & -18.63 & -8.19 \\
R_{\text{abs}}(H34) & -0.55 & -25.16 & -8.58 \\
R_{\text{abs}}(–H28/29/30) & 0.061 & -31.94 & -9.19 \\
R_{\text{abs}}(–H8/H10) & -0.05 & -19.89 & -9.19 \\
R_{\text{abs}}(–H23/H24) & 0.22 & -40.91 & -9.35 \\
\end{array}
\end{align*}
\]
electron, for example, \( f_k^+ \) designates the capability of a reactive center in a neutral molecule (\( N \) electrons) to accept an electron and pass to the ionized form (\( N+1 \) electrons) and so on. Consequently, the calculated values reflect the tendency of a specific site to undergo a nucleophilic, electrophilic, or radical attack. The Fukui indices for neutral and ionized cephalixin in gas and aqueous phases are calculated with Eqs. (1), (2), and (3); the results are summarized in Tables 1, 2, 3, and 4, respectively.

The Fukui indices were determined to predict the initial attack position during the photocatalytic degradation by reactive oxygen species (ROS). According to Table 1 (in gas phase), the most positive atoms with higher values of \( f_k^+ \) function are localized at C25 (0.46), C31 (0.04), and C40 (0.01). However, in aqueous phases (Table 2), C14 (0.07), H24 (0.17), and H21 (0.12) atoms showed the more positive values of \( f_k^+ \) function (atom abstraction by •OH). Consequently, the addition of •OH maybe took place on these atoms (nucleophilic attack). Nevertheless, the most negative part of \( f_k^- \) function in gas phase is localized in C25 (−0.14), C26 (−0.31), and C40 (−0.30), whereas C14 (−0.12), C25 (−0.39), C26 (−0.29), and C40 (−0.15) are regarded as the most favorable sites for electrophilic attack in aqueous phases. In the case of ionized cephalixin in gas phases (Table 3), the atoms with more positive values of \( f_k^+ \) function are localized in C26 (0.13) and C39 (0.03). However, C25 (0.23) and C26 (0.10) atoms in aqueous phases (Table 4) showed the most positive values and the best negative part of \( f_k^- \) function is localized in C26 (−0.68) and C31 (−0.10).

The reaction sites extracted from the analysis of atom condensed Fukui indices are circled (in red) and labeled, as shown in Figs. 4 and 5. To facilitate the recognition of the reaction site in future studies, abstraction sites are assigned and labeled with numbers (from (1) to (11)), and additional sites are labeled with letters (from (a) to (e)), as shown in Figs. 6 and 7.

The free energies, enthalpies, and radical stabilization energies of the abstraction and addition radicals are calculated with Eqs. (7), (9), and (10), and the results are displayed in Tables 5, 6, and 7, respectively. The most stable abstraction radical corresponds to \( R_{abs}^5 (−H38) \) with the highest stabilization energy (−0.08 kcal/mol). The \( R_{abs}^3 \) (−H9) and \( R_{abs}^2 \) (−H8/H10) radicals have almost the same stability, although the generation of the \( R_{abs}^3 \) (−H9) radical is more energetically favorable (para position) than that of H8 and H10 (meta position). The abstraction of an H proton from positions 10 (−H34) and 4 (−H13) is found to be more favorable than the abstraction of an H proton from the benzene ring; this is explained on the basis of the stabilization generated by the conjugation of the carboxyl radical and the benzyl radical in the second and third cases. The generation of these three radicals, \( R_{abs}^5, R_{abs}^3, \) and \( R_{abs}^2, \) is exergonic and thermodynamically favorable.
compared to other abstraction reactions. Generation of the radical $R_{\text{abs}1}$ (−1.16) has a free energy value very close to the values obtained for $R_{\text{abs}3}$ (−1.12) and $R_{\text{abs}2}$ (−1.12). Also, it has a similar RSE value. The abstraction radical $R_{\text{abs}9}$ (−H28/29/30) is the most stable radical after the three preceding radicals; its formation is accompanied by energy absorption (endergonic), and its stability is also explained by conjugation with the double bond (allylic radical).

The $R_{\text{abs}11}$ (−H20), $R_{\text{abs}7}$ (−H21), and $R_{\text{abs}8}$ (−H23/H24) radicals are the least stable. Their formations are the most endergonic, and their low stability is explained by the absence of stabilizing effects (conjugation and hyperconjugation) next to the radical.

In general, in the gas phase, abstraction radicals are more stable than those produced in the aqueous phase. The most stable addition radical is that corresponding to $R_{\text{add}}^a$ (C31) with a stabilization energy of 0.02 kcal/mol. This radical seems to be more unstable by moving from the neutral form CLX to the anionic form CLX− (−0.94 kcal/mol).

The $R_{\text{add}}^a$ (C25) radical is the least stable. Its formation is the most endergonic (0.08 kcal/mol), and its low stability is explained by the fact that the center of the radical is adjacent to the carboxyl group which exerts an inductive attracting effect on it (destabilizing effect). On the other hand, the addition of the OH radical to the C25 carbon ($R_{\text{add}}^a$ (C25)) generates a more stable tertiary radical (because of the inductive donor effect of the three groups that surround it).

The addition of the OH radical to C14 ($R_{\text{add}}^c$ (C14)) and C40 ($R_{\text{add}}^d$ (C40)) ends with the breaking of the amide bond (hydrolysis reaction) which is aliphatic in the first case and cyclic in the second case [16, 29]. The resistance of the cyclic amide to the 4-chain link is weaker than that of the aliphatic amide, resulting in a high tendency to react, i.e., −27.73 kcal/mol compared to 9.85 kcal/mol.

**Fragmentation CLX/CLX−**

The fragmentation of CLX/CLX− is modeled to predict the possible fates of the generated radical intermediates and to predict the most likely degradation pathways. All fragmentation pathways are shown in Fig. 8. The reaction energies of all optimized fragments are calculated using Eq. (10) and presented in Table 8 in ascending order. Cleavage of the benzylamine moiety (entry 1) appears to be easier than the

### Table 8 Fragmentation free energies of CLX/CLX− in gas and aqueous phase using DFT/B3LYP/6-31+G(d)

| Entry | CLX (kcal/mol) | CLX−(kcal/mol) | CLX aqueous phase (kcal/mol) | CLX− aqueous phase (kcal/mol) |
|-------|----------------|----------------|-----------------------------|-----------------------------|
| Entry 1 (− benzylamine) | 46.56 | 43.29 | 45.8 | 44.11 |
| Entry 2 (desamination (− NH₂)) | 53.59 | 53.96 | 52.71 | 52.08 |
| Entry 3 (− CH₃) | 53.59 | −319.4 | 79.69 | −296.1 |
| Entry 4 (− phenyl) | 66.51 | 62.43 | 60.8 | 60.42 |
| Entry 5 (− benzylaminecarbamide) | 75.17 | −303.08 | 72.79 | −300.07 |
| Entry 6 (− COOH/− COO−) | 76.74 | 71.84 | 76.56 | 64.5 |
| Entry 7 (− benzylaminecarbonyl) | 77.56 | 75.61 | 74.05 | 75.17 |
| Entry 8 (− OH/− O−) | 87.6 | 139.18 | 86.22 | 110.19 |
other fragmentation pathways. In the gas phase, deamination (entry 2) and demethylation (entry 3) have the same difficulty. Moreover, the losses of (OH) (entry 8) in neutral form and (O−) in anionic form are more endergonic than those of the others. The decarboxylation is less endergonic for CLX− (71.84 (64.5) kcal/mol) than for CLX (76.74 (76.56) kcal/mol). The reactions in the aqueous phase are, in general, less endergonic than those in the gas phase (solvent effect).

Fig. 9  Proposed mechanism of cephalexin degradation
In the ionized form of cephalexin, demethylation (entry 3) and the loss of benzylamine carbamide (entry 5) are the most exergonic and appear to have almost the same spontaneity. The eliminations of benzylamine carbonyl (entry 7) and benzylamine carbamide (entry 5) and decarboxylation (entry 6) are very close in energy. According to Hammond’s postulate [30], the structure of the transition state is similar for these three reactions. The negative values of Gibbs free energy ($\Delta G < 0$) mean that the pathway was exergonic processes (the release of energy from the reaction), indicating that the hydroxyl radical $\cdot$OH adding to this reaction site P3 (Fig. 9) may be more favored by thermodynamics; in addition, the cephalexin molecule was ruptured (Entry 5) and then formed a series of stable products, namely P4, P5, and P6.

**Proposed mechanism of degradation**

In this part, we are concerned with supporting our thermodynamic study by mechanistic research taking into account the abstraction, addition, and CLX/CLX$^-$ fragmentation radicals previously presented in this work. Figure 9 shows the mechanism suggested for the degradation of cephalexin to generate byproducts (P1–P7) which are identified experimentally by liquid chromatography–mass spectrometry (LC–MS) [2, 16, 29].

The degradation byproduct P1 is formed by opening the cyclic amide after the addition of the OH radical to give the intermediate $R_{\text{add}}^c$, and the degradation reaction progresses to fragmentation (removal of OH) to form P2 or decarboxylation to form P3. Analogic degradation pathways have been reported in previous studies for the degradation of cephalosporins and amoxicillin [31, 32]. The byproduct P3 reacts with the OH radical at C14 (hydroxylation reaction) passing to the $R_{\text{add}}^d$ radical followed by removing NH$_2$ (deamination) to generate P7. The other route is fragmentation (removal of benzylamine carbamide) to form P4, which ends in dehydration to form P5 and fragmentation (removal of OH) to form P6. The transformation pathway based on the theoretical results is well correlated with the previous experimental findings reported in the literature based on the MS spectra and m/z values of byproducts of photocatalytic degradation [2, 16, 17].

**Toxicity estimation**

The ecotoxicity of CLX and its byproducts to aquatic organisms was estimated at three trophic levels (fish, *Daphnia*, and green algae) based on the European Union evaluation criteria for new chemical substances. The octanol–water partition coefficient (log $K_{ow}$) was predicted to be 0.4 for CLX, and this value is in good correlation with that reported in the literature obtained experimentally (0.65) [28, 33].

| Toxicity range (mg/L) | Classification |
|-----------------------|----------------|
| LC$_{50}$/EC$_{50}$ ≤ 1 | Very toxic |
| 1 < LC$_{50}$/EC$_{50}$ ≤ 10 | Toxic |
| 10 < LC$_{50}$/EC$_{50}$ ≤ 100 | Harmful |
| LC$_{50}$/EC$_{50}$ > 100 | Non toxic |

**Table 9** Global harmonized system’s classification of toxicity using ECOSAR software

| Toxicity range (mg/L) | Classification |
|-----------------------|----------------|
| LC$_{50}$/EC$_{50}$ ≤ 1 | Very toxic |
| 1 < LC$_{50}$/EC$_{50}$ ≤ 10 | Toxic |
| 10 < LC$_{50}$/EC$_{50}$ ≤ 100 | Harmful |
| LC$_{50}$/EC$_{50}$ > 100 | Non toxic |

| Toxicity values (LC$_{50}$ and EC$_{50}$) of CLX/CLX$^-$ and its degradation byproducts to aquatic organisms (mg/L) via ECOSAR |
|----------------|----------------|----------------|
|                | Green algae (EC$_{50}$) | Daphnia (LC$_{50}$) | Fish (LC$_{50}$) |
| CLX/CLX$^-$    | 879              | 738              | 7460            |
| P1             | 5820             | 3790             | 43500           |
| P2             | 513              | 1010             | 976             |
| P3             | 6520             | 4110             | 48000           |
| P4             | 10100            | 5550             | 69600           |
| P5             | 223000           | 77800            | 1230000         |
| P6             | 272              | 694              | 537             |
| P7             | 42.3             | 162              | 491             |

[30] Springer
The toxicities of degradation byproducts were classified according to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) [28], as shown in Table 9. The toxicity was measured for the degradation byproducts (P1–P7) as well as the starting molecule (cephalexin); the results are presented in Table 10.

The toxicity of degradation byproducts was examined for the three tropic levels (fish, Daphnia and green algae). For green algae, 4 degradation byproducts (P1, P3, P4, and P5) are less toxic than CLX, and P2, P6, and P7 are more toxic. In the case of Daphnia, 5 degradation byproducts (P1, P2, P3, P4, and P5) are less toxic than CLX, and P6 and P7 are more toxic. For fish, 4 degradation byproducts (P1, P3, P4, and P5) are less harmful than CLX, and P2, P6, and P7 are more toxic.

As degradation continues, the toxicity worsens; furthermore, the degradation byproducts P1, P3, P4, and P5 are less toxic than cephallexin to all three organisms, while the byproducts P2, P6, and P7 are always more toxic than cephallexin, except for Daphnia, where P2 is less toxic. For green algae, all byproducts have an LC50/EC50 > 100 mg/L and are classified as nonharmful, with the exception of P7, which has an EC50 of 42.3 and is classified as harmful. CLX and most of the byproducts were found to be safe to aquatic organisms, and P7 is the only byproduct that is harmful to Daphnia. As a consequence, ZnO nanowire photocatalytic oxidation is a safe and clean process and is encouraged in the treatment of wastewater contaminated by cephallexin.

**Conclusion**

In this study, theoretical scale degradation of cephallexin was investigated in terms of reactivity, degradation thermodynamics, mechanism, and ecotoxicological impact of degradation byproducts. It was revealed that the affinity of cephallexin toward addition reactions increases as it passes from the gas phase to aqueous phase and from the neutral to ionized form and decreases in the same way for the abstraction reactions. Thermodynamic data showed that the most favorable abstraction and addition radicals formed are those in which the reactant site is weakened by the inductive withdrawing effect, and the most stable radicals formed are those possessing larger mesomeric forms. The degradation byproducts (P1–P7) identified experimentally in the photocatalytic degradation of CLX by ZnO nanowires under simulated sunlight were verified in this computational work. The ecotoxicological impact of the degradation byproducts was clarified with ECOSAR software, where toxicity analysis revealed that CLX/CLX− was not toxic. Only its degradation product P7 was harmful, and the other oxidation byproducts (P1, P3, P4, and P5) were less toxic to fish and green algae. At the same time, P1, P2, P3, P4, and P5 were not toxic to Daphnia, and the rest of the byproducts were more toxic than cephallexin but still can be classified as nontoxic to the three organisms. Based on the collected information about the byproduct toxicities, we conclude that the photocatalytic degradation of cephallexin is a safe process for ecological organism studies. This study is the first to assess the safety of a photocatalytic process for the degradation of cephallexin based on a DFT approach toward the intermediate and byproduct structures of the degradation process.

**Author contribution** R. Masmoudi: investigation, methodology, writing—original draft, writing—reviewing and editing. S. Khettaf: methodology, validation. A. Soltani: formal analysis, validation. A. Dibi: conceptualization, validation. L. Messaadia: software, formal analysis. M. Benamira: supervision, validation, writing—reviewing and editing.

**Funding** This study was financially supported by the General Directorate of Scientific Research and Technological Development (DGDRSTD) of Algeria through the national research program (PRFU N° B00L01UN180120220001).

**Data availability** The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Code availability** Gaussian 16 program package: G16W Full Version, 32-Bit, Rev: A.03 Front: 1.1. Version: Multiprocessor. GaussianView6 for Windows, Rev: 6.0.16, 32-bit. V32108462166828W-4627. N. ECOSAR Class Program (Free Ware).

**Declarations**

**Competing interests** The authors declare no competing interests.

**References**

1. Bailey A, Walker A, Hadley DG (1971) James, Cephalexin. A new oral antibiotic, Torax 20:15–19
2. He J, Zhang Y, Guo Y, Rhodes G, Yeom J, Li H, Zhang W (2019) Photocatalytic degradation of cephallexin by ZnO nanowires under simulated sunlight: kinetics, influencing factors, and mechanisms. Environ Int 132:105105. https://doi.org/10.1016/j.envint.2019.105105
3. Estrada AL, Li Y-Y, Wang A (2012) Biodegradability enhancement of wastewater containing cefalexin by means of the electro-Fenton oxidation process. J Hazard Mater 227:41–48. https://doi.org/10.1016/j.jhazmat.2012.04.079
4. Benarab N and Fangninou FF (2020) The issues of Antibiotics: Cephalexin Antibiotic as emerging environment contaminant. Int J Sci Res Publ 10:306–318. https://doi.org/10.29322/IJSRP.10.2020.p9843
5. Ikehata K, JodeiriNaghashkar N, Gamal El-Din M (2006) Degradation of aqueous pharmaceuticals by ozonation and advanced oxidation processes: a review, Ozone Sci. Eng. 28:353–414. https://doi.org/10.1080/01919510600985937
6. Mandal T, Maity S, Dasgupta D, Datta S (2010) Advanced oxidation process and biotreatment: their roles in combined industrial
wastewater treatment. Desalination 250:87–94. https://doi.org/10.1016/j.desal.2009.04.012
7. Stefan MI (2017) Advanced oxidation processes for water treatment: fundamentals and applications. IWA publishing. https://doi.org/10.2166/9781780407197_0297
8. Kanakaraju D, Glass BD, Oelgemöller M (2018) Advanced oxidation process-mediated removal of pharmaceuticals from water: a review. J Environ Manage 219:189–207. https://doi.org/10.1016/j.jenvman.2018.04.103
9. Cao J, Ren Q, Chen F, Lu T (2015) Comparative study on the methods for predicting the reactive site of nuleophilic reaction. Sci China Chem 58:1845–1852. https://doi.org/10.1007/s11426-015-5494-7
10. Yang W, Mortier WJ (1986) The use of global and local molecular parameters for the analysis of the gas-phase basicity of amines. J Am Chem Soc 108:5708–5711
11. Drogueut C, Salazar R, Brillas E, Sirés I, Carlesi C, Marco JF, Thiam A (2020) Treatment of antibiotic cephalixin by heterogeneous electrochemical Fenton-based processes using chalcopyrite as sustainable catalyst. Sci Total Environ 740:140154. https://doi.org/10.1016/j.scitotenv.2020.140154
12. Bansal P, Verma A (2018) N, Ag co-doped TiO2 mediated modified in-situ dual process (modified photocatalysis and photo-Fenton) in fixed-mode for the degradation of cephalixin under solar irradiances. Chemosphere 212:611–619. https://doi.org/10.1016/j.chemosphere.2018.08.120
13. Wu H, Feng Q, Yang H, Alam E, Gao B, Gu D (2017) Modified biochar supported Ag/Fe nanoparticles used for removal of cephalixin in solution: characterization, kinetics and mechanisms. Colloids Surfaces A Physicochem Eng Asp 517:63–71. https://doi.org/10.1016/j.colsurfa.2017.01.005
14. Perea LA, Palma-Goyes RE, Vazquez-Arenas J, Romero-Ibarra I, Ostos C, Torres-Palma RA (2019) Efficient cephalixin degradation using active chlorine produced on ruthenium and iridium oxide anodes: role of bath composition, analysis of degradation pathways and degradation extent. Sci Total Environ 648:377–387. https://doi.org/10.1016/j.colsurfa.2018.08.148
15. Coledadam DAC, Pupo MMS, Silva BF, Silva AJ, Egiluz KIB, Salazar-Banda GR, Aquino JM (2017) Electrochemical mineralization of cephalixin using a conductive diamond anode: a mechanistic and toxicity investigation. Chemosphere 168:638–647
16. Antonín VS, Aquino JM, Silva BF, Silva AJ, Rocha-Filho RC (2019) Comparative study on the degradation of cephalixin by four electrochemical advanced oxidation processes: evolution of oxidation intermediates and antimicrobial activity. Chem Eng J 372:1104–1112. https://doi.org/10.1016/j.cej.2019.04.185
17. Guo W, Wang H, Shi Y, Zhang G (2010) Sonochemical degradation of the antibiotic cephalixin in aqueous solution. Water Sa. 36. https://doi.org/10.4314/wssv.36i5.61998
18. Alalm MG, Tawfik A, Ookawara S (2015) Degradation of four pharmaceuticals by solar photo-Fenton process: kinetics and costs estimation. J Environ Chem Eng 3:46–51
19. Becke AD (1996) Density-functional thermochemistry. IV. A new dynamical correlation functional and implications for exact-exchange mixing. J. Chem. Phys. 104:1040–1046. https://doi.org/10.1063/1.470829
20. Ho J, Ertem MZ (2016) Calculating free energy changes in continuum solvation models. J Phys Chem B 120:1319–1329. https://doi.org/10.1021/acs.jpcb.6b00164
21. Yamana T, Tsuji A (1976) Comparative stability of cephalosporins in aqueous solution: kinetics and mechanisms of degradation. J Pharm Sci 65:1563–1574. https://doi.org/10.1002/jps.2600651104
22. Roy KK, Pal S, Hirao K (1999) On non-negativity of Fukui function indices. J Chem Phys 110:8236–8245. https://doi.org/10.1063/1.478792
23. Galano A, Alvarez-Idaboy JR (2009) Guanosine + OH radical reaction in aqueous solution: a reinterpretation of the UV-Vis data based on thermodynamic and kinetic calculations. Org Lett 11:5114–5117. https://doi.org/10.1021/ol901862h
24. An T, Gao Y, Li G, Kamat PV, Peller J, Joyce MV (2014) Kinetics and mechanism of •OH mediated degradation of dimethyl phthalate in aqueous solution: experimental and theoretical studies. Environ Sci Technol 48:641–648
25. Sanderson H, Johnson DJ, Wilson CJ, Brain RA, Solomon KR (2003) Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECO-SAR screening. Toxicol Lett 144:383–395. https://doi.org/10.1016/S0378-4274(03)00257-1
26. Al-Musawi TJ, Kamani H, Bazzafshan E, Panahi AH, Silva MF, Abi G (2019) Optimization the effects of physicochemical parameters on the degradation of cephalixin in sono-Fenton reactor by using Box–Behnken response surface methodology. Catal Letters 149:1186–1196
27. Legnoverde MS, Simonetti S, Basaldella El (2014) Influence of pH on cephalixin adsorption onto SBA-15 mesoporous silica: theoretical and experimental study. Appl Surf Sci 300:37–42
28. Cinar SA, Ziylan-Yavaş A, Catak S, Ince NH, Aviyente V (2017) Hydroxyl radical-mediated degradation of diclofenac revisited: a computational approach to assessment of reaction mechanisms and by-products. Environ Sci Pollut Res 24:18458–18469. https://doi.org/10.1007/s11356-017-9482-7
29. Tavasol F, Tabatabaie T, Ramavandi B, Amiri F (2020) Design a new photocatalyst of sea sediment/titanate to remove cephalixin antibiotic from aqueous media in the presence of sonication/ultraviolet/hydrogen peroxide: Pathway and mechanism for degradation. Ultrason Sonochem 65:105062. https://doi.org/10.1016/j.ultraschon.2020.105062
30. Farcausiu D (1975) The use and misuse of the Hammond postulate. J Chem Educ 52:76. https://doi.org/10.1021/ed052p76
31. Guerra MMH, Alberola IJ, Rodriguez SM, López AA, Merino AA, Alonso IMQ (2019) Oxidation mechanisms of amoxicillin and paracetamol in the photo-Fenton solar process. Water Res 156:232–240
32. Hsu MH, Kuo TH, Chen YE, Huang CH, Hsu CC, Lin AYC (2018) Substrate reactivity affecting the manganese dioxide oxidation of cephalosporins. Environ Sci Technol 52:9188–9195. https://doi.org/10.1021/acs.est.8b02448
33. K. Kümerer, Pharmaceuticals in the environment: sources, fate, effects and risks, Springer Science & Business Media, 2008.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.