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Degradation of cephalosporins in aqueous solutions by UVC, UVC/H_2O_2 and UVC/PS

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Abstract The present study was focused on the degradation of three cephalosporins, namely cefuroxime, cefotaxime, and ceftazidime, in aqueous solutions under UVC (i.e., λ = 254 nm) irradiation, either alone or in the presence of two oxidants, namely H_2O_2, and persulfate (PS). The objective of this study was to assess the performance of the above treatment processes for the degradation of cephalosporins in aqueous matrices. It was found that the studied treatment methods were not only extremely efficient for the degradation of target compounds in aqueous matrices but also they achieved a significant decrease in the total organic carbon concentration when the oxidants were applied.

Keywords: UVC/H_2O_2, UVC/PS, cefuroxime, cefotaxime, ceftazidime

1. Introduction

In recent years, more emphasis has been given on the monitoring of pharmaceutical residues in the aquatic environment. Indeed, several pharmaceutical compounds can frequently end up in waste water treatment plants (WWTPs) effluents and surface waters, at concentrations up to several $\mu g \cdot L^{-1}$.
Cephalosporins are a widely used class of β-lactam antibiotics in human and veterinary medicine and they are detected at significant concentrations at the inlet and outlet of WWTPs (Hirsch et al., 1999, Kummerer 2009). The present study was focused on the degradation of three cephalosporins, namely cefuroxime, cefotaxime and ceftazidime, in aqueous solutions under UVC (i.e., λ = 254 nm) irradiation, either alone or in the presence of two oxidants, namely H_2O_2 and $S_2O_8^{2-}$ (persulfate, PS).

2. Materials and Methods

2.1. Chemicals and reagents

The experiments were conducted using cefuroxime sodium salt ($C_{16}H_{15}N_4NaO_8S$) purchased from Sigma-Aldrich, cefotaxime sodium salt ($C_{16}H_{16}N_5NaO_7S_2$) and ceftazidime ($C_{22}H_{22}N_6O_7S_2$), both purchased from Tokyo Chemical Industry- TCI. Phosphate salts, such as $Na_2HPO_4 \cdot H_2O$ and KH_2PO_4 (both obtained from Merck) were employed for the preparation of aqueous buffer solutions. Sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$) was obtained from Sigma-Aldrich and acetonitrile was purchased from Merck and both were used as solvents for high performance liquid chromatography (HPLC). All aqueous solutions were prepared with ultrapure water from the purification system Simplicity UV supplied by Millipore.
Stock solutions of cephalosporins were prepared by dissolving the appropriate amount of each cephalosporin in the buffer solution. The exact concentration of the resulting aqueous stock solutions was measured by HPLC.

2.2. UVC-irradiation experiments

UVC irradiation was provided by an 11 W, low pressure mercury lamp (Philips TUV, PL-S, G23), which emits predominately at 254 nm. UVC irradiation experiments were conducted in an immersion well, batch type, laboratory scale photochemical reactor. It consists of an inner cylindrical quartz glass vessel, housing the lamp (length: 250 mm, outer diameter: 36 mm) and an external double-walled, cylindrical, borosilicate glass, reaction vessel (length: 230 mm, internal diameter: 63 mm, volume capacity: 450 mL). The reaction mixture was placed in the external cylindrical reaction vessel and the inner cylindrical quartz glass vessel was immersed inside the reaction mixture. The UVC lamp was placed inside the inner cylindrical quartz glass. The external double-walled,

cylindrical, borosilicate glass, reaction vessel was effectively cooled by a water circulation stream through the double-walled compartment, acting as a cooling water jacket. During photolysis experiments, temperature was maintained constant at 22±2ºC. This reaction geometry is ideal for full exploitation of the UVC irradiation emitted from the lamp. In a typical photolysis run, 450 mL of the aqueous solution containing the desired concentration of cephalosporin was loaded in the reaction vessel and a measured volume of the concentrated H_2O_2 solution or $Na_2S_2O_8$ solution was added in the reaction mixture to achieve the desired concentration of each oxidant in the reaction mixture. Immediately after the addition of the reaction mixture, the UVC lamp was placed inside the inner quartz vessel of the photochemical reactor and the reaction mixture was continuously stirred with a magnetic stir bar and a magnetic stirrer. Unless otherwise stated, the reaction mixture was exposed to ambient air and no further aeration took place.

2.3. Analytical Methods

Cefuroxime concentration was determined at 273 nm, cefotaxime concentration was determined at 235 nm and ceftazidime concentration was determined at 255 nm using HPLC (Alliance 2695 Waters with a Diode Array Detector) and a Kinetex 2.6 μm C18 column purchased from Phenomenex (150 mm×4.6 mm). The mobile phase was a mixture of phosphate buffer 20 mM and acetonitrile using a gradient program with a flow rate of 0.5 $mL \cdot min^{-1}$. The gradient started with 90% phosphate buffer and this composition was held constant till the 6th minute. After the 6th minute the composition of phosphate buffer proceeded to 80% till the 7th minute and then returned to the initial conditions for a further 2 minutes. The temperature of the column compartment was 30 °C, and the injected volume for HPLC analysis was 100 μL.

H_2O_2 concentrations were determined using a spectrometric method at 410 nm and $Na_2S_2O_8$ concentrations were also determined by using a spectrometric method at 352 nm. Total organic carbon (TOC) concentration was measured on a Shimadzu 5000A TOC analyzer whose operation is based on non-dispersive infra red gas analysis.

3. Results & Discussion

Cephalosporins possessing an alkox-imino group, $R_2O{-}N{=}C$, at the sidechain of Δ^3-cephem ring exist either in syn (or *Z*) and anti (or *E*) isomer. However, it has been found that syn (or *Z*) isomers of these cephalosporins exhibit superior antibacterial activity compared to the corresponding anti (or *E*) isomers (Lerner et al., 1988; Fabre et al., 1992; Fabre et al., 1994). Figure 1, 2, and 3 shows the syn(*Z*)-isomer and anti(*E*)-isomers of cefuroxime, cefotaxime and ceftazidime.

In an initial set of photolysis experiments, the direct photolysis, under UVC irradiation of cephalosporins was studied at an initial concentration of 9 $\mu mol \cdot L^{-1}$ and at pH 7. The results are shown in Figures 5, 6, and 7. UVC irradiation was able to degrade all cephalosporins in a rather short period of time. In addition, TOC concentration was measured, and it was found that the TOC of these solutions remained practically constant after 60 min irradiation.

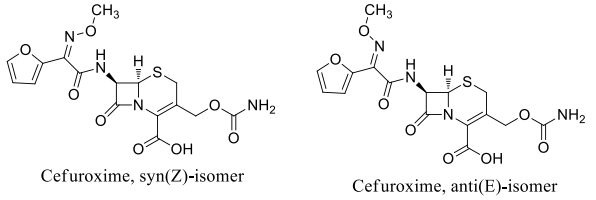

Figure 1. Geometrical isomers of cefuroxime: syn(Z)-isomer and anti(E)-isomer.

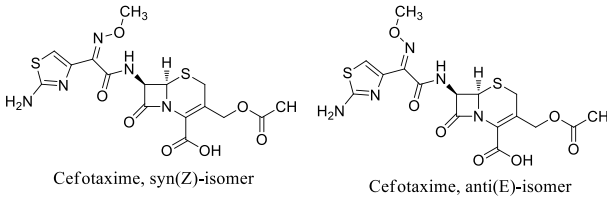

Figure 2. Geometrical isomers of cefotaxime: syn(Z)-isomer and anti(E)-isomer.

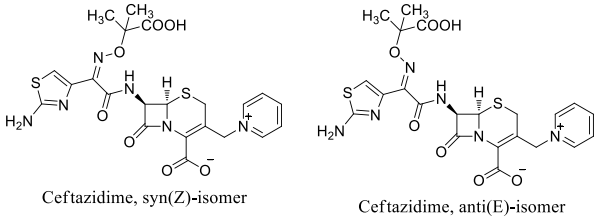

Figure 3. Geometrical isomers of ceftazidime: syn(Z)-isomer and anti(E)-isomer.

The reactivity of these compounds under UVC irradiation is the result of two competitive processes, namely: (i) isomerization and (ii) photolysis (Figure 4). The photochemical reactions of cefuroxime, cefotaxime and ceftazidime are attributed to the isomerization of syn(*Z*)-isomer to anti(*E*)-isomer and the photochemical opening of the β-lactamic ring and the Δ^3-cephem ring (Figure 4).

More specifically, the direct photolysis of cefuroxime (Figure 5) under UVC radiation was relatively fast and it was found that after 120 s of radiation the degradation of cefuroxime was approximately 99%. It is obvious that the anti(*E*)-isomer of cefuroxime was formed as soon as the irradiation proceeds. Its concentration goes through a maximum at 20 s and then drops mainly because of the photochemical decomposition process.

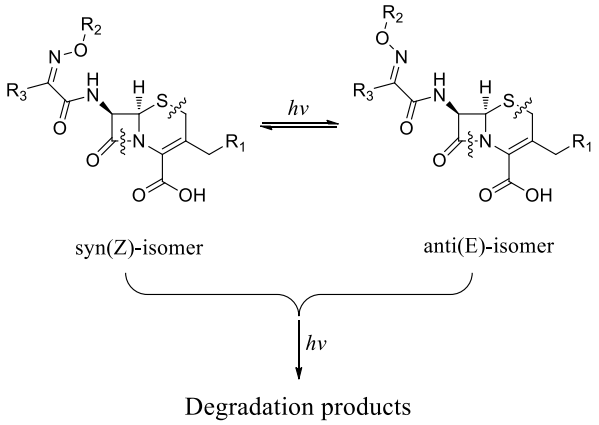

Figure 4. Photochemical reactions of cephalosporins.

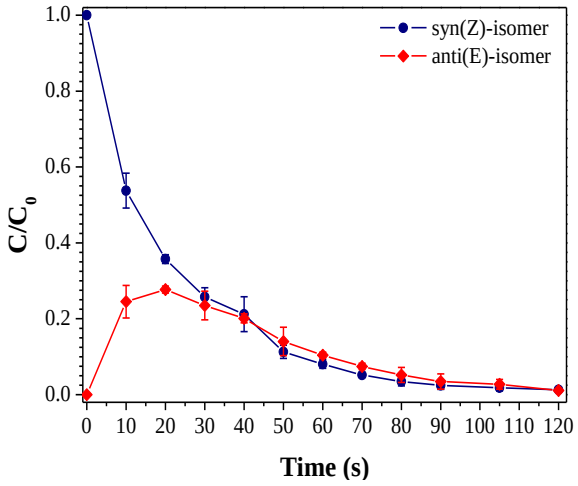

Figure 5. Direct UVC photolysis of cefuroxime. Experimental conditions: $[Cefuroxime]_0$ = 9 μmol·L^{-1}, phosphate buffer pH = 7.

In addition, the same trend has been observed in the case of cefotaxime (Figure 6). The maximum concentration of anti(*E*)-isomer of cefotaxime was monitored at 30 s while after 90 s of irradiation the total decrease of cefotaxime was 95%. In the case of ceftazidime (Figure 7) the concentration of anti(*E*)-isomer reached a maximum at 20 s while the concentration of syn(*Z*) isomer of ceftazidime decreases relatively slowly compared to cefuroxime and cefotaxime. In fact, it was found that the degradation of the syn(*Z*) isomer of ceftazidime was 90% after 45 minutes of radiation.

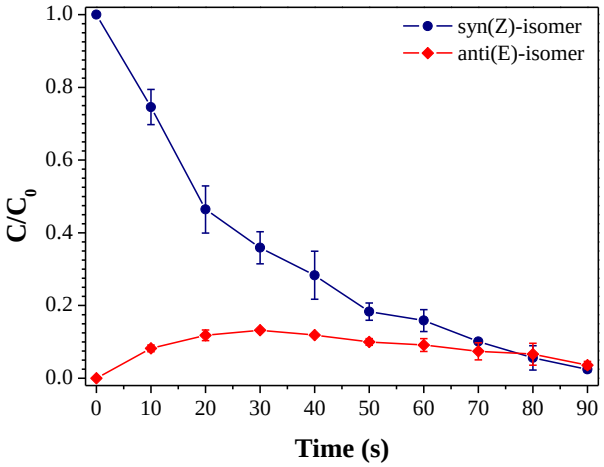

Figure 6. Direct UVC photolysis of cefotaxime. Experimental conditions: $[Cefotaxime]_0$ = 9 μmol·L^{-1}, phosphate buffer pH = 7.

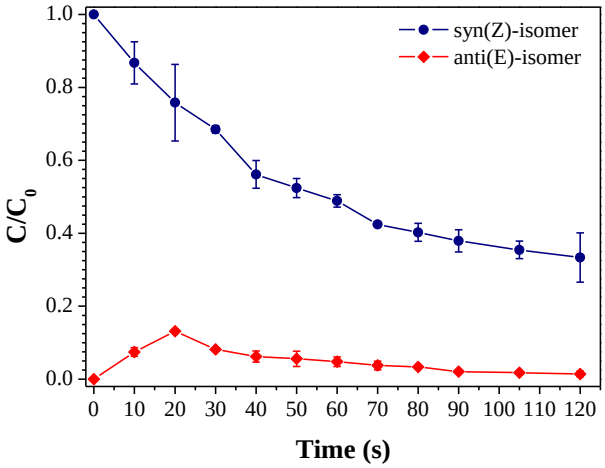

Figure 7. Direct UVC photolysis of ceftazidime. Experimental conditions: $[Ceftazidime]_0$ = 9 μmol·L^{-1}, phosphate buffer pH = 7.

However the degradation of cephalosporins was not accompanied by any reduction in the concentration of the total organic carbon of the solution. Consequently, it was concluded that the direct photolysis of cephalosporins lead to the formation of transformation products which were relatively stable and were not further degraded to mineral end-products.

For this reason, further photolysis experiments of cephalosporins were conducted under UVC radiation in the presence of H_2O_2 or $S_2O_8^{2-}$. It is well known that upon illumination under UVC radiation of H_2O_2 or $S_2O_8^{2-}$, the peroxide O–O bond of both molecules undergoes homolytic cleavage from its electronically excited state, thus leading to the formation of hydroxyl and sulphate radicals. In further experiments, the photochemical degradation of cephalosporins was studied under UVC irradiation in the presence of either H_2O_2 or $S_2O_8^{2-}$. More specifically, the concentration of

H_2O_2 or $S_2O_8^{2-}$ was 90 μmol·L^{-1} (yielding 3.06 mg·L^{-1} H_2O_2 or 17.3 mg·L^{-1} $S_2O_8^{2-}$), while the initial concentration of each cephalosporin was 9 μmol·L^{-1} in ultrapure water and at pH 7. The results for cefuroxime, cefotaxime and ceftazidime are shown in Figures 8, 9 and 10.

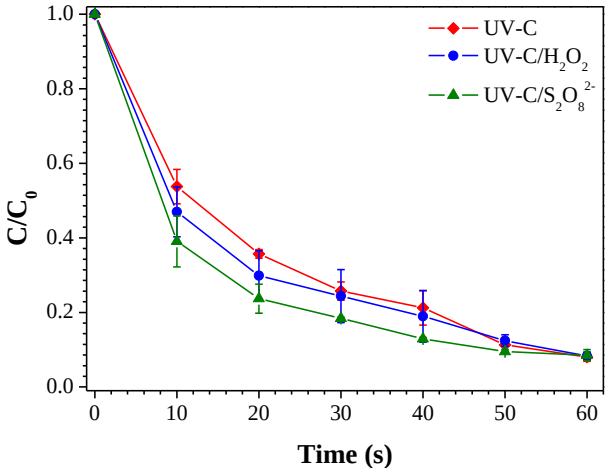

Figure 8. Direct UVC photolysis, UVC/H_2O_2 and UVC/$S_2O_8^{2-}$ photochemical oxidation of cefuroxime.

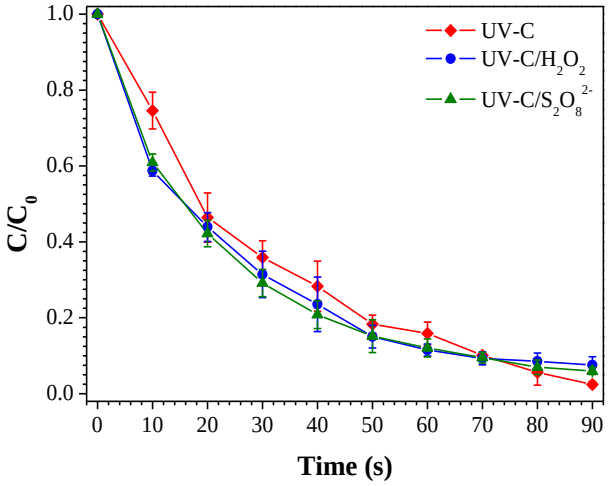

Figure 9. Direct UVC photolysis, UVC/H_2O_2 and UVC/$S_2O_8^{2-}$ photochemical oxidation of cefotaxime.

As can be seen in the Figures 8, 9 the addition of oxidants didn't enhance significantly the total removal of cefuroxime and cefotaxime. However the photochemical removal of ceftazidime was increased with the addition of H_2O_2 and $S_2O_8^{2-}$ compared to the direct UVC photolysis (Figure 10). Therefore, it was found that after 10 minutes of irradiation in the presence of H_2O_2 or $S_2O_8^{2-}$ the degradation of ceftazidime was 98%. In the presence of H_2O_2 or $S_2O_8^{2-}$ the overall degradation of cephalosporins was attributed to both direct and indirect photolysis mainly due to hydroxyl or sulphate radicals produced during the process.

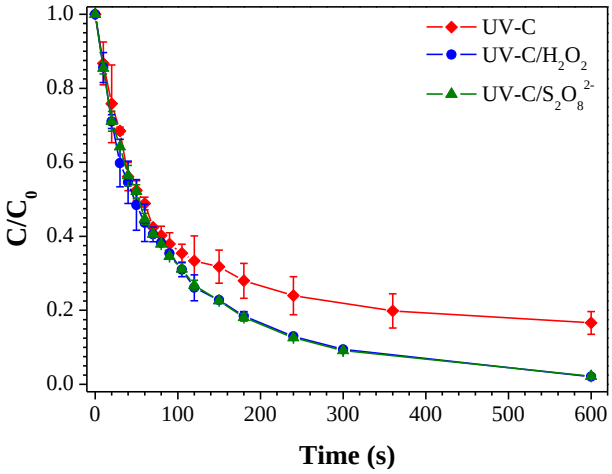

Figure 10. Direct UVC photolysis, UVC/H_2O_2 and UVC/$S_2O_8^{2-}$ photochemical oxidation of ceftazidime.

To assess the effect of the addition of H_2O_2 or $S_2O_8^{2-}$ on the concentration of TOC, further experiments were conducted with initial concentration of each cephalosporin at 22.4 μmol·L^{-1}, while the concentration of H_2O_2 or $S_2O_8^{2-}$ was 1.5 mM, 3 mM and 15 mM. It was found that the addition of oxidants enhanced the removal of TOC. In the case of H_2O_2, the percentage of TOC removal was in the range from 19 to 30% for cefuroxime, from 10 to 24% for cefotaxime and from 13 to 21% for ceftazidime. The highest removal of total organic carbon was achieved by the addition of $S_2O_8^{2-}$. In that case the observed removals were in the range from 46 to 57% for cefotaxime and from 41 to 71% for ceftazidime.

4. Conclusions

Overall, the above results indicate that UVC irradiation. either alone or in the presence of both oxidants, at the present experimental conditions, was very efficient for the degradation of the three cephalosporins. At the same time, the TOC concentration of the solutions weas substantially decreased, especially in the case of UVC/$S_2O_8^{2-}$. However, none of the studied methods was able to achieve total mineralization, thus showing the formation of relatively stable photochemical transformation products, which are resistant to any further photochemical degradation.

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