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EVALUATION OF PHOTOCHEMICAL DEGRADATION OF ANTIVIRAL ACYCLOVIR BY UVC/PS & UVC/H_2O_2 IN SYNTHETIC FRESH AND HYDROLYZED URINE

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Abstract

The increasing presence of contaminants of anthropogenic origin in the aquatic environment raises the concern of the scientific community (Patel et al., 2019). Antiviral drugs play an important role, as their presence in aquatic bodies is associated with antiviral resistance and chronic toxicity to aquatic organisms (Nannou et al., 2020). Acyclovir is one of the widest antivirals as it is preferred for the treatment of various diseases such as herpes simplex virus, hepatitis virus, and cytomegalovirus. Acyclovir has been detected in WWTPs and industrial effluents as well as drinking water. In the present study, the UVC/PS and UVC/H_2O_2 treatment of ACV was investigated in synthetic fresh (SFU) and hydrolyzed urine (SHU). Specifically, the effect of each component of these complex aqueous matrices on the performance of the treatment methods was evaluated. It was found that UVC/H_2O_2 system is effective in ACV decomposition even SHU and SFU, as almost complete degradation of the antiviral is achieved in a short time of treatment. In contrast, UVC/PS system presents a lower efficiency in ACV degradation. It was found that the presence of urea in SFU and NH_3 in SHU, inhibits the photo-generated sulfate radicals and consequently significantly slows down the decomposition of antiviral.

Keywords: *Acyclovir, antiviral, AOPs, UVC/H_2O_2, UVC/PS, fresh urine, hydrolyzed urine*

1. Introduction

In recent years, more than 700 emerging pollutants, their metabolites, and their transformation products have been recorded in effluents of WWTPs, in rivers, lakes, and groundwater (Geissen et al., 2015). These pollutants, known as contaminants of emerging concern (CECs), include compounds that are released into the environment and can potentially have a negative impact on ecosystems and public health. These are mainly organic substances which include pharmaceuticals, hormones, food additives, personal care products (PCPs), and others compounds (Geissen et al., 2015, Tang et al., 2019). Among pharmaceuticals, antivirals play an important role as they are detected in the wastewater. Their limited biodegradability classifies them as hazardous compounds causing chronic toxicity to aquatic organisms as well as to humans (Gupta et al., 2021). Acyclovir (ACV) belongs to the class of antivirals and specifically to the category of antiherpetics. It is a widespread drug as it is prescribed for the treatment of a wide spectrum of viral diseases such as herpes simplex virus (HSV), varicella-zoster virus (VZV), hepatitis B virus (HBV), and cytomegalovirus (Gupta et al., 2021, Nannou et al., 2020). Only 15-20% of administered acyclovir is metabolized in the human body and the rest is discharged through human excretion in the urban wastewater (Gupta et al., 2021). ACV and its major transformation product (TPs), carboxyl-ACV, have been detected in several aquatic systems like WWTPs and industrial effluents, surface, and groundwaters in concentrations levels which are ranging from ng/L to a few mg/L (Gupta et al., 2021, Nannou et al., 2020).

Among the streams entering into the WWTPs, the possibility of separating the source of urine is a very promising prospect for the effective removal of pharmaceuticals, as urine carries a significant portion of drugs entering the aquatic environment (Zhang et al., 2016). For this reason, it is important to develop new effective methods, which can degrade drug substances in the urine. In recent years, Advanced Oxidation Processes, AOPs, have been considering promising methods for the degradation of pharmaceuticals. Their performance is based on the *in situ* generation of active radicals, such as hydroxyl radicals ($^{\bullet}OH$) and sulfate radicals ($SO_4^{\bullet-}$) (Zhang et al., 2016).

In the present study, the photochemical degradation of Acyclovir was investigated. More specifically, the performance of UVC/PS and UVC/H_2O_2 systems on acyclovir degradation was studied in synthetic fresh (SFU) and hydrolyzed urine (SHU). Moreover, the effect of each substrate of these water matrices on AOPs performance was investigated.

2. Experimental

2.1. Materials and methods

Acyclovir ($C_8H_{11}N_5O_3$, MW=225.208 g·L^{-1}, ACV, 98%, CAS No 59277-89-3) was purchased from Carbosynth (Compton, Berkshire, UK). H_2O_2 as a 30 % w/w aqueous solution was purchased from Sigma-Aldrich, while sodium persulfate ($Na_2S_2O_8$, PS, 98%, CAS No: 7775-27-1) was purchased from Alfa Aesar. Chemical reagents used for the preparation of synthetic fresh and hydrolyzed human urine were as follows: urea (Merck-Millipore), NaCl (Penta Chemicals), Na_2SO_4 (Sigma-Aldrich), KCl (Merck-Millipore), ammonium hydroxide solution (≥25% NH_3 basis, Sigma-Aldrich), $MgCl_2$•$6H_2O$ (Scharlau), NaH_2PO_4·H_2O (Sigma-Aldrich), $CaCl_2$•$2H_2O$ (Pfaltz & Bauer), NH_4HCO_3 (Sigma-Aldrich), and $Na_3Citrate$•$2H_2O$ (Alfa Aesar). Moreover, $NaHCO_3$ and concentrated H_3PO_4 (85 wt. % in H_2O) were purchased from Sigma-Aldrich. Phosphate salts, such as Na_2HPO_4·$2H_2O$ and KH_2PO_4 (both obtained from Merck-Millipore) were employed for the preparation of aqueous buffer solutions. All the reagents were used as received. Methanol used for high-performance liquid chromatography (HPLC) (LiChrosolv grade) was purchased from Merck-Millipore. Ultrapure water (UPW, pH = 5.5 and resistivity 18.2 MΩ·cm at 25 ºC), employed for solution preparation and as an HPLC eluent. Aqueous solutions of ACV were prepared in four matrices, namely: (i) phosphate-buffered solutions at various pH values (PB); (ii) synthetic fresh human urine (SFU), (iii) synthetic hydrolyzed human urine (SHU) and ultrapure water (UPW). SFU and SHU were prepared according to the literature (Zhang et al., 2016).

2.3. Photolysis of ACV in the presence of H_2O_2 or $S_2O_8^{2-}$ (PS) under UVC radiation (λ_{max}=254nm)

In a typical experimental run, 450 mL of ACV aqueous solution dissolved in the appropriate aqueous matrix containing the desired initial concentration of the antiviral, were loaded into the reaction vessel. Then, the appropriate amount of hydrogen peroxide (H_2O_2) or sodium persulfate ($Na_2S_2O_8$) was added to the reaction mixture to achieve the desired initial concentration of the oxidant. In continuous, the UVC lamp (a low-pressure mercury lamp, Philips TUV, PL-S, G23, 11W, λ_{max}=254 nm) which was switched on outside the photochemical reactor for approximately 15 min to stabilize its photon emission, was placed inside the inner quartz sleeve of the photochemical reactor and the reaction mixture was continuously stirred with a magnetic stir bar and a magnetic stirrer. Samples were taken at regular intervals to quantify the residual antiviral. Each experiment was performed twice.

2.4. Analytical methods

The determination and quantification of ACV, was performed by high-performance liquid chromatography (Waters Alliance 2695 HPLC system), coupled with a diode array detector (Waters 2996 PDA detector), employing a Luna C18 column (5 μm, 4.6 mm × 250 mm) and a C18 security guard column (4 mm × 3 mm), both purchased from Phenomenex. The mobile phase was a mixture of an aqueous PB (0.010 mol·L^{-1} at pH = 5.0, solvent A) and methanol (solvent B), in gradient elution at a flow rate of 1 ml·min^{-1}, with injection volume 50 μL. The column was thermostated at 30 ºC, while ACV was detected by the diode array detector set at 252 nm. Under these conditions, the retention time was 5.5 min.

3. Results

3.1. Effect of SFU and SHU on UVC/H_2O_2 system

UVC/H_2O_2 experiments were carried out in SHU and SFU matrices. The initial concentration of ACV was 50 μM while the H_2O_2 concentration was 20 mM. The results of the above experiments are presented in Figure 1 (b). For comparison purposes, the figure also includes experiments performed in PB solutions (10 mM) at the respective pH values of SFU and SHU (i.e., at pH 6.0 and 9.1, respectively). In addition, Table 1 presents the corresponding values of the initial rate, r_0. As shown in Figure 1 (b), in the case of SHU, the initial rate of photochemical degradation of ACV was slightly reduced compared to the corresponding experiments performed at the same pH value (i.e., r_0 was 0.37± 0.07μmol·L^{-1}·s^{-1} and 0.42±0.01μmol·L^{-1}·s^{-1}, respectively).

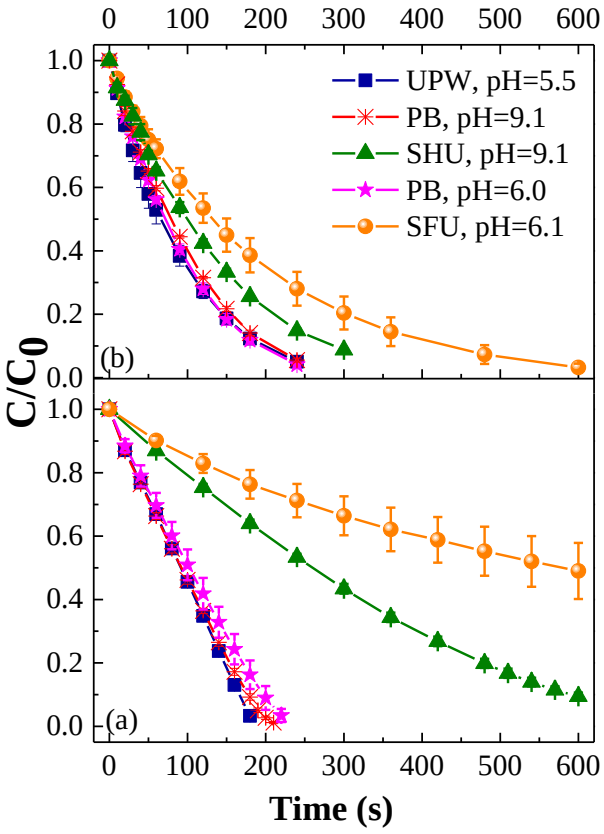

Figure 1. ACV degradation by (a) UVC/PS and (b) UVC/H_2O_2 systems in UPW, SFU, SHU, and PB solutions at corresponding pH values. Experimental Conditions: [ACV]=50 μM, [H_2O_2] =20 mM, [PS]=2.5 mM

The above results suggest that (a) the pH value of the aqueous matrix does not affect the ACV degradation and (b) the components of SHU, such as NH_4^+ and HCO_3^-, probably react with the photo-formed •OH radicals, causing a slight reduction of r_0. However, photochemical degradation of the antiviral in SHU by UVC/H_2O_2 is considered satisfactory as the observed ACV degradation is more than 90% after five minutes of irradiation. In the case of SFU, the pH of the aqueous matrix does not affect the ACV degradation. In addition, the rate of photochemical degradation of ACV was reduced by almost 50% compared to the corresponding experiments at the same pH value (i.e., the initial rate, r_0, was 0.27±0.04 and 0.48±0.02 μmol·L^{-1}·s^{-1}, respectively). Indeed, as shown in Figure 1, ten minutes of irradiation are required to achieve almost 95% ACV removal. In this case, the components of SFU, such as urea and citrate anions, react significantly with the photo-formed hydroxyl radicals, resulting in a relative decrease of the rate of photochemical degradation of ACV. From the above, it can be concluded that the UVC/H_2O_2 method is quite effective in the decomposition of ACV even in relatively complex aqueous matrices such as SHU and SFU as almost complete decomposition of the antiviral is achieved in a short time of treatment.

Table 1. Initial rate, r_0, of photochemical degradation of ACV in UVC/H_2O_2 system in SFU and SHU. Experimental Conditions: $[ACV]_0$=50μM, $[H_2O_2]_0$= 20mM.

Water matrix	pH	r_0 (μmol·L^{-1}·s^{-1})
UPW	5.5	0.51±0.06
SFU	6.0	0.27±0.04
SHU	9.0	0.37±0.07
PB	6.0	0.48±0.02
PB	9.0	0.42±0.01

Table 2. Initial rate, r_0, of photochemical degradation of ACV in UVC/PS system in SFU and SHU. Experimental Conditions: $[ACV]_0$=50 μM, του $[PS]_0$= 2.5mM.

Water matrix	pH	r_0 (μmol·L^{-1}·s^{-1})
UPW	5.5	0.31±0.01
SFU	6.0	0,05±0,01
SHU	9.0	0,11±0,01
PB	6.0	0.28±0.02
PB	9.0	0.30±0.01

3.2. Effect of SFU and SHU on UVC/PS process

In continue, UVC/PS experiments were carried out. The initial concentration of ACV and PS was 50 μM and 2.5 mM, respectively. The results of the above experiments are shown in Figure 1(a). Similarly, for comparison purposes, the figure includes experiments which performed at PB solutions, at pH values of SFU, and SHU. In addition, Table 2 shows the corresponding initial rate, r_0. As can be seen in Figure 1 (a), a decrease in the initial rate of photochemical degradation of ACV, in SHU, was observed compared to the corresponding experiments performed at the same pH value in PB (i.e., the values of r_0 were 0.11±0.003 and 0.30±0.01 μmol·L^{-1}·s^{-1}, respectively). The above results suggest that the various components of SHU, react with the photo-formed sulfate radicals, resulting in the decrease of r_0 of ACV photochemical degradation (as demonstrated in the following sections). However, even in this case, it is observed ACV degradation more than 90% after 10 minutes of irradiation. In contrast, in the case of SFU, the rate of photochemical degradation of ACV was significantly reduced compared to the corresponding experiments performed at the same pH value (i.e., r_0 was 0.05 ± 0.01 and 0.28 ± 0.02 μmol ·L^{-1}·s^{-1}, respectively). Indeed, as shown in Figure 1 (a), ACV degradation is almost 50% after ten minutes of irradiation. In this case, the organic components contained in the SFU react significantly with the photo-formed sulfate radicals, resulting in a significant reduction in the rate of photochemical decomposition of the ACV (verified in the following sections). From the above results, it appears that the photochemical degradation of ACV in the UVC/PS system is negatively affected by complex aqueous matrices such as is the SHU and SFU. For this reason, further photochemical experiments were performed, by adding, individually, components of SFU and SHU, at the same concentrations contained in them, to investigate their effect on the performance of the UVC/PS system.

3.2.1 Effect of urea in SFU

Considering that in SFU the main component is urea, CH_4N_2O, its effect on the photochemical degradation of ACV via the UVC/PS system was studied. Therefore, experiments were performed in PB solutions, 10 mM, with an initial concentration of ACV, PS, and CH_4N_2O, 50 μM, 2.5 mM, and 0.25 M, respectively. The results are presented in Figure 3. For comparison purposes, the results of the experiments in UPW and PB at the corresponding pH value are included. As shown in Figure 3, ACV removal is almost 25% after ten minutes of irradiation, demonstrating the strong effect of urea on the photochemical decomposition of antiviral. This practically means that urea reacts with the photo-formed

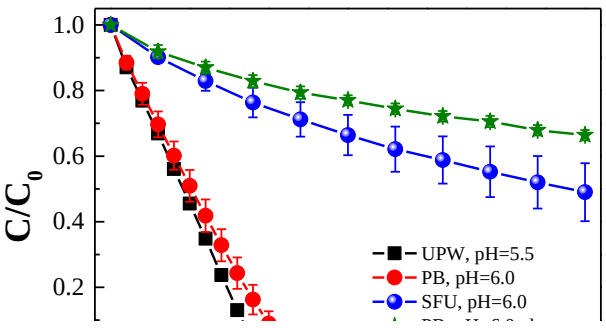

Figure 3. Effect of urea on ACV degradation by UVC/PS treatment. Experimental Conditions: [ACV]=50 μM, [PS]= 2.5mM

sulfate radicals to a significant degree (the reaction rate constant of urea with sulfate radicals is $k_{SO_4}^{\bullet-} = 6.35\times10^5$ $M^{-1}\cdot s^{-1}$, Zhang et al., 2015), resulting in a negative effect on the performance of the UVC/PS system.

3.2.2 Effect of Cl^-, HCO_3^- and NH_3 in SHU

In SHU, Cl^-, NH_3, and HCO_3^- are the main components. Therefore, additional experiments were performed to investigate their effect on ACV photochemical degradation. Specifically, further experiments were performed on PB, with a concentration of 10 mM and pH = 9.0 in the presence of NH_3, Cl^-, HCO_3^- and NH_4HCO_3 at concentrations of 0.5 M, 0.1 M, 0.25 M, and 0.25 M, respectively. The initial concentration of ACV was 50 μM while the concentration of PS was 2.5 mM. The results are presented in Figure 4. For comparison purposes, the results of experiments in UPW and PB in corresponding pH value are included.

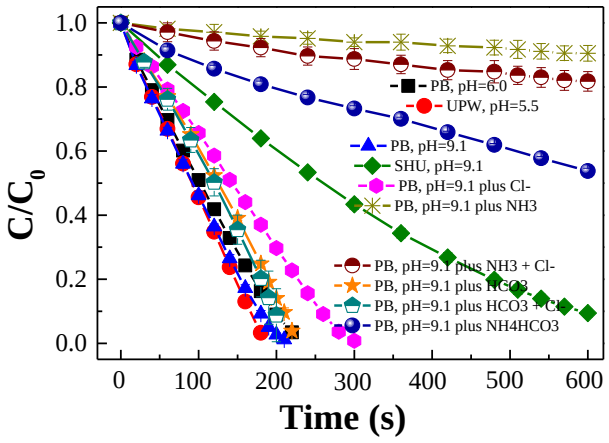

Figure 4. Effect of Cl^-, HCO_3^- and NH_3 on ACV degradation by UVC/PS treatment. Experimental Conditions: [ACV]=50 μM, [PS]= 2.5 mM.

As can been seen in Figure 4, the presence of NH_3 almost inhibits the action of the sulfate radicals. In particular, in the presence of NH_3, only 20% ACV degradation is observed after 10 minutes of irradiation.

4. Conclusions

In the present study, the photochemical degradation of ACV by UVC/H_2O_2 and UVC/PS systems, in synthetic fresh and hydrolyzed urine, was evaluated. It was found that in both systems a satisfactory degradation of the antiviral was achieved. More specifically, in the case of UVC/H_2O_2 system, although the rate of ACV decomposition decreases due to the scavenging of the hydroxyl radicals from the components of the aqueous matrices, however, almost complete decomposition of acyclovir (>90%) is observed in about 10 minutes of treatment. On the other hand, the UVC/PS system seems to be influenced to a greater extent by the components of the aqueous matrices. Specifically, it was found that urea in the case of SFU and NH_3 in the case of SHU, are the components that scavenging the sulfate radicals, reducing significantly the efficiency of the system. Nevertheless, the UVC/H_2O_2 and UVC/PS systems are promising methods for the removal of pharmaceuticals from aqueous matrices.

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