

Suspended and Immobilized TiO₂ Photocatalytic Degradation of Estrogens: Potential for Application in Wastewater Treatment Processes

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This work evaluated the photocatalytic activity of suspended TiO₂, and TiO₂-coated glass Raschig rings with respect to degradation of estrogens estrone (E1), 17 β -estradiol (E2), and 17 α -ethinylestradiol (EE2) in aqueous solutions and wastewater effluent samples. Gas chromatography/tandem mass spectrometry (GC-MS/MS) studies were carried out to identify degradation products for EE2. TiO₂ suspensions allowed degradation rates higher than 90% for all studied estrogens in 30 min for an artificial (ultraviolet A) UVA-assisted process and aqueous solution. Immobilized TiO₂ showed less favorable degradation kinetics, requiring ca. 60 min to achieve almost complete estrogen degradation. Some derivatized degradation products were identified for the first time by GC-MS/MS applying ultraviolet C (UVC) photolysis and TiO₂/UVA photocatalysis. A degradation route was suggested for EE2 in UVC photolysis and TiO₂/UVA processes. In the degradation of pretreated sewage samples, the immobilized TiO₂ was more efficient than free TiO₂, allowing ca. 85% removal of E2 and EE2 in 60 min.

Keywords: hormones, photocatalysis, immobilized TiO₂, wastewater, degradation products

Introduction

In the last twenty years, the high degradation capacity of advanced oxidative processes (AOPs) toward recalcitrant chemical species, including emergent contaminants such as estrogens, has been widely demonstrated.¹⁻³ In the context of AOPs, considerable emphasis should be given to TiO₂-mediated heterogeneous photocatalysis because of the excellent results achieved in environmental remediation.⁴

However, the need for separation systems⁵ and the restricted penetration of ultraviolet (UV) light in suspended photocatalyst systems offsets its well-known benefits and reduce its potential applicability to wastewater treatment.⁶ In addition, recent studies have demonstrated significant cytotoxic effects of nanosized anatase TiO₂.⁷

The use of immobilized photocatalysts has been proposed as a method to overcome these difficulties since 1993.⁵ Immobilization involves the use of inert supports, such as glass,^{8,9} activated carbon,^{10,11} silica-based materials,^{12,13} and polymeric materials.¹⁴

As highlighted by Carbonaro *et al.*,¹⁵ many studies have demonstrated the high degradation efficiency of TiO₂ toward organic micropollutants under ideal laboratory conditions.¹⁵ However, there are relatively few studies concerning photocatalyst performance in more complex matrices, such as wastewater effluent.¹⁶⁻¹⁸

Recently, degradation of endocrine disrupting compounds in both simulated and real municipal wastewater treatment plant effluents was evaluated using TiO₂ immobilized on glass rings.¹⁸⁻²⁰ In general, 85% of the target compounds were degraded within 120 min radiation time, indicating the utility of the system for the treatment of polluted water.¹⁸ TiO₂ was immobilized on glass microscope slides and employed in a benchtop continuous-flow reactor for the degradation of pharmaceutical micropollutants in a biologically treated wastewater effluent (WWE). The results demonstrated low degradation capacity of the TiO₂ photocatalysis in WWE samples, which was due to the presence of organic and inorganic hydroxyl radical (HO•) scavengers.¹⁵

To the best of our knowledge, there are few reports in the literature concerning photocatalytic degradation of

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estrogens by immobilized TiO_2 in effluents from municipal wastewater treatment plants.²¹ Hence, the main objective of this work was to investigate the degradation of estrone (E1), 17 β -estradiol (E2), and 17 α -ethinylestradiol (EE2) in aqueous solutions and wastewater using TiO_2 -coated glass Raschig rings. Moreover, gas chromatography/tandem mass spectrometry (GC-MS/MS) studies were carried out to better understand the mechanism pathway.

Experimental

Reagents and chemicals

Standard E1, E2, and EE2 reference reagents were purchased from Sigma-Aldrich (Steinheim, Germany) at > 97% purity. Standard stock solutions for the estrogens at the concentration level of 100 mg L⁻¹ were prepared in methanol, and stored in a freezer at -18 °C and protected from light. All diluted working solutions were prepared using deionized water (Milli-Q system, Millipore, Bedford, MA, USA).

In heterogeneous photocatalytic processes, TiO_2 (Degussa P-25, 75% anatase/25% rutile, BET 50 m² g⁻¹) was used.

Derivatization grade *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) and trimethylsilylimidazole (TMSI) were from Sigma-Aldrich (Steinheim, Germany). The derivatization mixture MSTFA was prepared containing 1% TMSI (v/v).

Immobilization of TiO_2 on borosilicate Raschig rings

The impregnation of borosilicate Raschig rings with TiO_2 was carried out according to the procedures described by Yeber *et al.*²² Borosilicate glass rings (5 mm in diameter and 2 mm in length) were cleaned with distilled water containing an anionic detergent and subsequently washed exhaustively with ultra-pure water. They were then immersed in acetone for 10 min and dried at 100 °C.

The TiO_2 -coated glass rings were prepared by immersing the rings in a 5% (m/v) solution of titanium (IV) butoxide prepared in ethanol and in an inert atmosphere for 45 min, followed by calcination step in air at 450 °C for 45 min. This immobilization process was repeated four times.

Photocatalytic treatment

Initially, photolytic and photocatalytic processes involving artificial radiation were carried out at the lab scale using a 250 mL cylindrical glass photoreactor equipped

with a magnetic stirrer and a water bath, which was used to maintain the operating temperature at 25 ± 2 °C. UV radiation was provided by a high-pressure mercury vapor lamp (125 W), immersed in the samples by means of a quartz (for UVC) or glass (for UVA) jacket.

The UV photon flux measured by uranyl oxalate actinometry was 9.7×10^{-5} mol of photon L⁻¹ s⁻¹.²³ The UV irradiation was 11 mW cm⁻² (UVC) and 22 mW cm⁻² (UVA). Samples were placed in the reactor and irradiated for different times, using a photocatalyst mass and a working pH according previously optimized by 2² factorial design.²⁴ The pH of the samples was adjusted by addition of diluted aqueous solutions (HCl or NaOH). Samples were collected at regular intervals and submitted to analytical control.

Degradation by immobilized photocatalyst was performed with 150 mL of estrogens (20 $\mu\text{g L}^{-1}$) solution and glass rings in sufficient amount to completely fill the reactor. The experiment was carried out under constant atmospheric air supply, provided by an aquarium pump, bubbled to the bottom of the reactor. The degradation process was accomplished up to 60 min.

Analytical control

The degradation of the target estrogens in aqueous solution was assessed by high-performance liquid chromatography (HPLC) equipped with a diode-array detector (DAD, 197 nm) and fluorescence detector (FLD, $\lambda_{\text{ex}} = 282$ nm and $\lambda_{\text{em}} = 306$ nm), using a Varian 920-LC chromatograph equipped with an autosampler, quaternary gradient pump, and fluorescence detector (Agilent 1260). Routine chromatographic separations were performed on a Varian Microsorb C18 column (250 \times 4.6 mm, 5 μm), using a Metaguard pursuit 5 μm C18 (4.6 mm) pre-column. The mobile phase comprised acetonitrile:water (50:50) (v/v) and was used at a flow rate of 0.8 mL min⁻¹. Analytical curves were established between 0.010 and 1.000 mg L⁻¹ ($R^2 > 0.99$).

After photochemical treatment, the samples were vacuum filtered in a closed glass system using GF-3 0.60 μm filters (Carvalhoes, GF-3, Germany). When necessary, solid-phase extraction (SPE) of the selected compounds was performed with a PrepSep 20-port vacuum manifold (Waters, Milford, MA, USA), using Hypersep C18 500 mg cartridges (Thermo Scientific, USA) previously conditioned with successive additions of acetonitrile (7 mL), methanol (5 mL), and ultrapure water (5 mL). Aqueous samples (100 mL) were passed through the cartridges at 4 mL min⁻¹, and the solid phase was dried by a gentle flow of nitrogen and the estrogens were eluted with acetonitrile (5 mL). After being dried under nitrogen flow, the residue was resuspended in methanol (1 mL).²⁵

Under these conditions, mean recoveries of $97.8 \pm 4.9\%$, $98.0 \pm 2.0\%$, and $97.0 \pm 5.6\%$ were observed for E1, E2, and EE2 ($20 \mu\text{g L}^{-1}$), respectively.

GC-MS/MS analysis

Photolytic and TiO₂ photocatalytic degradation products were determined after derivatization in a gas chromatography (GC) System 7890A interfaced with mass spectrometer detector Triple Quadrupole 7000 (Agilent, Santa Clara, CA, USA). SLBTM-5ms capillary column ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$) from Supelco Analytical was employed. $1 \mu\text{L}$ of the samples were injected in splitless mode. The quadrupole analyzer, ion source, transfer line and injector temperatures were set at 150, 270, 280 and 290°C , respectively. The oven temperature was programmed to increase from 50 to 150°C at $30^\circ\text{C min}^{-1}$, increased to 300°C at $10^\circ\text{C min}^{-1}$ and held for 5 min. Total run time was 23.3 min. Helium was used as the carrier gas at 1 mL min^{-1} as flow rate. The filament delay was set to 5 min and the fragment ions were analyzed over 50-600 mass-to-charge ratio (m/z) mass range. Data acquisition and processing were performed with Agilent MassHunter Workstation software (qualitative analysis).

The intermediates of photolytic and photocatalytic degradation processes were extracted by liquid-liquid extraction with ethyl acetate ($3 \times 20 \text{ mL}$) and then evaporated to dryness by a rotary vacuum evaporator and after by gentle nitrogen stream. The sample extracts were derivatized by adding $30 \mu\text{L}$ of the derivatization mixture (MSTFA/1% TMSI). After a reaction time of 30 min at 60°C , the residue was dissolved in 1.0 mL of ethyl acetate. Samples were then analyzed by the GC-MS/MS.

Results and Discussion

Degradation by photolysis

Under the experimental conditions used in this and our previous work,²⁴ degradation by UVC photolysis is very significant for E1, allowing almost complete removal of the estrogens studied in exposure times lower than 10 min (Figure 1). In the presence of lower energy radiation (UVA), degradation of estrogens by photolysis is low, being significant only for E1, which exhibited ca. 25% degradation at 60 min (Figure 1). Estrogens have high absorptivity in the UV region and, among the estrogens, E1 is more susceptible to degradation by photolysis ($\lambda < 365 \text{ nm}$).^{26,27} For E2 and EE2, UVA photolysis was negligible (degradation below 2%) meanwhile the same behavior of E1 was observed applying UVC radiation.²⁴

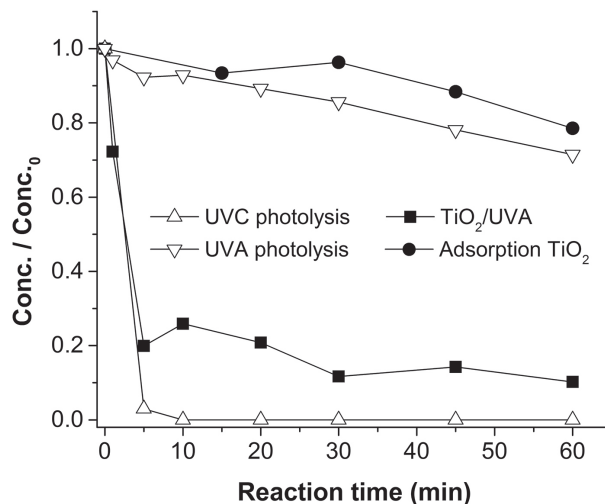


Figure 1. UVC and UVA photolytic degradation of E1, removal by adsorption on the TiO₂ photocatalyst and, benchtop UVA photocatalytic degradation (E1 concentration: $20 \mu\text{g L}^{-1}$, pH: 7.0-7.5, TiO₂: 250 mg L^{-1}).

These results are consistent with the observations of Liu and Liu,²⁶ who reported photolytic degradation of ca. 95 and 60% for E1 and E2, respectively, under UVC radiation ($\lambda = 254 \text{ nm}$, 30 W). Additionally, it was observed that the photolysis process occurs more readily at low estrogens concentrations and at higher pH values (> 5).²⁶

As pointed out by Lin and Reinhard,²⁸ the strong absorption of UVB radiation allows the rapid photolytic degradation of E1 at an irradiation intensity of 250 W m^{-2} ($\lambda = 290\text{--}700 \text{ nm}$), with a typical half-life of ca. 5 h. This behavior is related to the presence of the carbonyl group, which can be excited to its triplet state.²⁸

Considering that the estrogens degradation under UVC radiation was fast and very similar, EE2 was chosen to evaluate the degradation products produced after 5, 15 and 30 min of reaction time. In the UVC photolytic degradation of EE2, four major peaks were observed after 5 min of reaction (Figure 2). According to the National Institute of Standards and Technology (NIST) library, peak II m/z 440, retention time (t_R) = 18.4 min, corresponds to di-trimethylsilyl derivative of EE2 with similarity of 93.1%. The fragmentation pattern in MS/MS spectra is analogous to that observed by Zhou *et al.*,²⁹ with characteristic mass fragments 425, 285 and 196. Peaks III and IV showed the same m/z value (527.2, corresponding to tri-trimethylsilyl derivative), but different retention times and mass spectra. The proposed structures were 2-hydroxylated-EE2 (2OH-EE2) to peak III and 6-hydroxylated-EE2 (6OH-EE2) to peak IV, taking into account the reactivity of HO• toward both unsaturated and saturated rings.³⁰⁻³² The structure of 2OH-EE2 has already been identified by HPLC-MS analysis.³³

According to Mazellier *et al.*,³³ monochromatic (254 nm) and polychromatic ($\lambda > 290 \text{ nm}$) irradiation

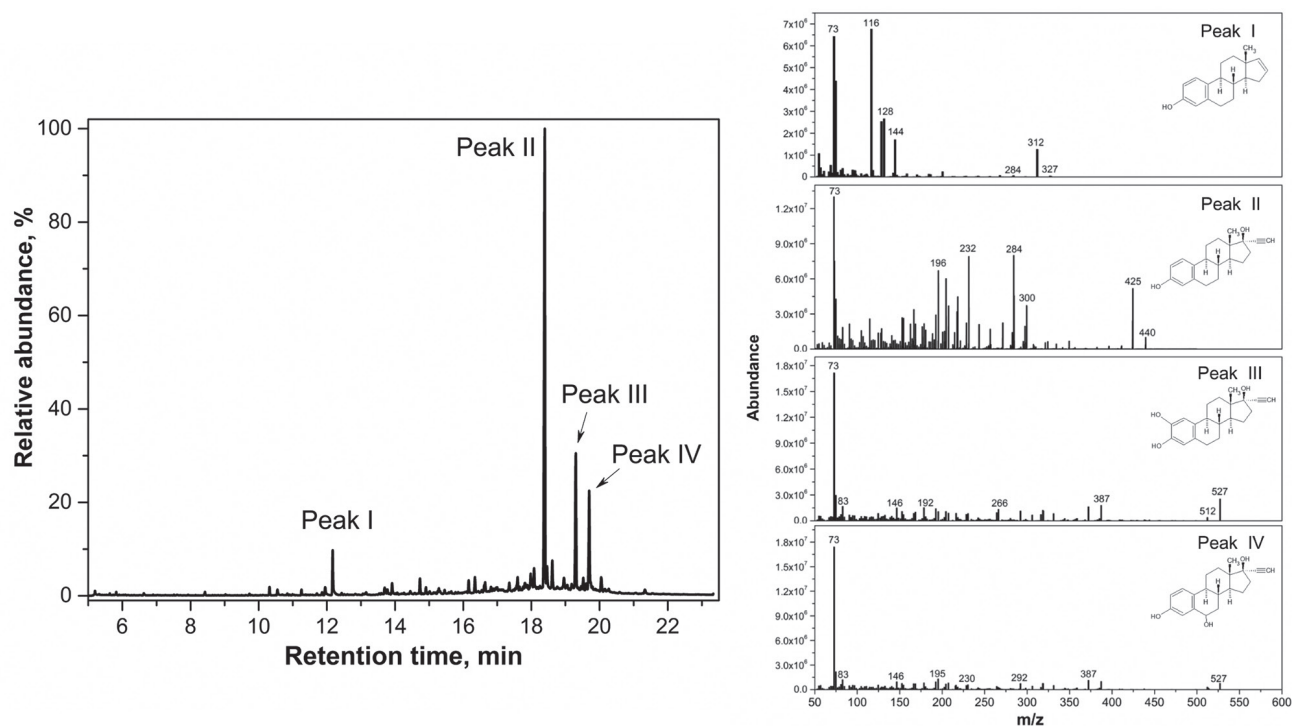


Figure 2. GC-MS analysis of degradation products after UVC photolytic degradation of 17 α -ethynylestradiol (EE2; exposure time: 5 min).

of EE2 leads to the formation of only one product, identified by GC-MS as a quinone methide derivative. This degradation product was not observed under the experimental conditions of this study. Nevertheless, GC-MS analysis confirmed the formation of several photoproducts, including monohydroxylated derivatives arising from hydroxylation in aromatic and unsaturated rings. 2OH-EE2 in derivatized form was also found by Ren *et al.*³⁰ by GC-MS during photodegradation of EE2 in aqueous solution. Carp *et al.*³² suggested that alpha carbon to the aromatic ring (C6) could be a reactive site to \bullet OH attack. Based on this condition, 6OH-EE2 can be plausibly generated during photolysis. To the best of our knowledge, the 6OH-EE2 in their derivatized form from UVC photolysis is being proposed for the first time.

Additionally, another photolytic degradation product eluted at 12.15 min (peak I) was found with m/z 326.8. According to the MS/MS spectra, UVC photolytic can lead to the formation of compound I after the loss of the hydroxyl and the ethynyl moieties at C17. The ethynyl group was cleaved, resulting in a double bond between C16-C17. This new binding favors loss of the hydroxyl of C17 position. Since this compound elutes before the derivatized EE2, the structure proposed agreed with the volatility increase. Furthermore, the fragmentation data exhibit m/z fragment related to the derivatized molecule (m/z 73). Despite the ethynyl moiety being the group with the lowest reactivity in the EE2 molecule,³⁴ other authors

have proposed similar structures to those found in this work.³⁵

For reaction times of 15 and 30 min, no degradation products were detected under the experimental conditions employed. This result may be related to an increase in the derivatized byproduct mass or the rapid degradation of estrogens under photolytic treatment.

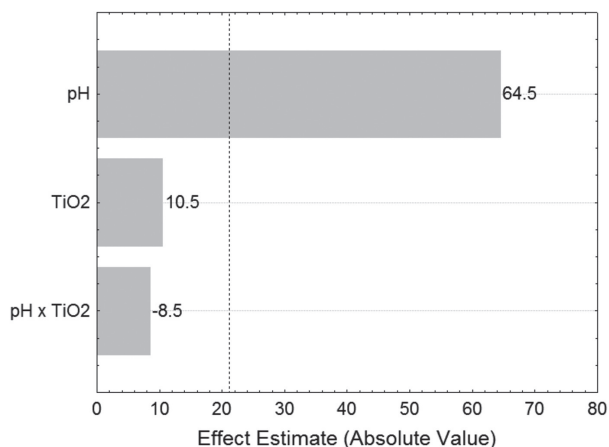
Degradation by suspended TiO₂ photocatalyst

Because of the partial degradation observed in photolytic processes assisted by UVA radiation and the impossibility of observing the heterogeneous photocatalytic process in the presence of strong UVC photolysis, subsequent studies were performed with artificial UVA radiation. TiO₂ photocatalytic degradation efficiency was previously investigated by 2² factorial design. The results of studies involving the degradation of E1 (Table 1), which represents typical behavior of the three estrogens studied, indicate a strong positive effect (64.5) of pH and a small positive effect (10.5) of the photocatalyst concentration, which suggests a maximum degradation efficiency under the conditions of experiment using mass and pH at level (+) (8 and 750 mg L⁻¹, respectively). Additionally, no second-order effect was observed, which implies a non-interaction effect of pH and TiO₂ concentration levels on the degradation of estrogens.

Despite that, a low effect of the TiO₂ concentration at high pH values (Figure 3A), and a significant positive effect

Table 1. Factors and levels studied in the 2² factorial design to evaluate the E1 degradation

Assay	Factor		E1 removal / %
	pH	TiO ₂ amount / mg	
1	4 (-)	250 (-)	7
2	8 (+)	250 (-)	80
3	4 (-)	750 (+)	26
4	8 (+)	750 (+)	82
5/6/7	6 (0)	500 (0)	20 ± 5

**Figure 3.** Pareto chart of effects of the 2² factorial design elaborated to study the effect of pH and photocatalyst concentration on the bench UVA photocatalytic degradation of estrone (E1 concentration: 20 µg L⁻¹, reaction time: 10 min).

at low pH values demonstrate the greater importance of the photocatalytic process, which is consistent with the low degradation efficiency of UVA radiation alone.

Additional degradation tests were performed at pH 7, which corresponds to the natural pH of treated sewage samples. Under this condition, degradations close to 80% were observed for the three estrogens studied at reaction times of 10 min. This result is very similar to those obtained at pH 8 and it was selected for further experiments at low level of TiO₂ catalyst (250 mg L⁻¹).

Numerous studies^{36,37} have discussed the influence of pH and the amount of catalyst on the degradation efficiency of photocatalytic processes. Usually, the effect of pH is related to its influence on the charge distribution on the photocatalyst surface and on the substrate protonation equilibria, characteristics that control the preliminary adsorption of the substrates on the catalyst surface. However, according to Bahnemann *et al.*,³⁸ the analysis of these effects is a difficult task, because of the number of different electrostatic attractions involving the semiconductor surface, solvent and substrate molecules, and radical species generated in the process.

With respect to the degradation of estrogens, Coleman *et al.*³⁶ reported significant improvement on the degradation of E2 when the pH was increased from 2 to 7, as well as a significant loss in efficiency between pH 7 and 10. Likewise, Zhang *et al.*³⁷ reported a significant enhancement on the degradation of E1 and E2 when the pH was increased from 2 to 7.6.

According to Bahnemann *et al.*,³⁸ the effect of the photocatalyst load is a function of several factors, including the radiation potency and the reactor geometry. The effect of the TiO₂ concentration on the photocatalytic degradation of E2 was studied by Mai *et al.*³⁹ using UVA radiation. They found that the efficiency of the degradation process increased when the amount of catalyst was increased from 0.1 to 0.5 g L⁻¹. Above this concentration range, a significant loss in the efficiency of the degradation process was observed.³⁹

Under TiO₂ photocatalysis optimized conditions, ca. 20% of the E1 and less than 5% of E2 and EE2 were adsorbed after a contact time of 60 min. Figure 1 shows the adsorption process for E1.

Regarding photocatalysis under UVA light, the degradation process occurred extremely fast in the first few minutes of reaction with ca. 90% of estrogens removal in 60 min. The results of the present study were consistent with the observations of Marinho *et al.*²⁴ which found similar results to E2 and EE2 at the same experimental conditions. Puma *et al.*⁴⁰ also showed similar degradation behavior for multicomponent mixture of estrogens (E1, E2, EE2 and E3). The percentage of degradation was between 92 and 97% after 120 min of reaction using a UVA lamp and pH 7.0.⁴⁰

Degradation by immobilized TiO₂

Photocatalysts supported on glass rings have been characterized in our previous study,²³ which was shown to contain ca. 15% (m/m) of the anatase polymorph of TiO₂ homogeneously distributed over the glass surface.

Degradation studies were carried out at pH 7.0-7.5, using 150 mL of a mixture of E1, E2, and EE2 (20 µg L⁻¹ each), and glass rings in sufficient quantity to completely fill the reactor (ca. 200 mg L⁻¹ of TiO₂). The photocatalytic process was applied for durations up to 60 min in the presence of UVA radiation, with continuous bubbling of atmospheric air with the aid of an aquarium pump. The results (Figure 4) show average degradations of ca. 98% for all the estrogens studied, and practically negligible removal by adsorption, at an exposure time of 60 min. The lower kinetic efficiency of the immobilized catalyst in relation to the free form can be attributed to some factors

as the lower contact surface of TiO_2 when in the supported form, amount of immobilized TiO_2 which was 20% lower than suspended TiO_2 , and the difference of solution stirring (performed by aeration).

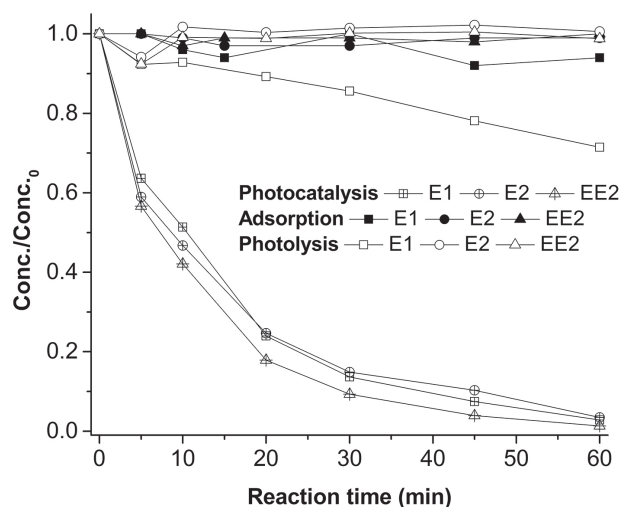


Figure 4. E1, E2 and EE2 removal by adsorption on the supported TiO_2 on Raschig rings and bench UVA photolysis and photocatalysis (estrogens: $20 \mu\text{g L}^{-1}$, pH: 7.0-7.5).

Coleman *et al.*³⁶ studied the photocatalytic degradation of E2 by immobilized TiO_2 in metallic alloy and 98% of the initial estrogen was degraded in 3.5 h. They also employed UVA radiation but the lamp was positioned out of estrogen solution, attenuating the degradation process. Also, Tanizaki *et al.*⁴¹ studied the photocatalytic degradation of E1, E2, and EE2 using a quartz beads coated with a thin film of TiO_2 . E2 was degraded ca. 90% in 120 min, while E1 and EE2 showed the same degradation rate in only 30 min.⁴¹ Independently of geometry reactor and the sort of immobilization, immobilized TiO_2 was suitable to degrade these substances with high removal percentages.

Also, both UVA photolytic degradation and removal by adsorption onto the support are practically negligible under the working conditions; thus, the observed degradation is promising, especially considering that the use of supported forms of the catalyst avoids subsequent complex removal processes.

Photocatalysis degradation pathways of EE2

The high estrogen degradation capacity of the photocatalytic process is not surprising, due to the remarkable number of organic substrates that can be quickly degraded by photocatalysis. However, it is important to point out that such degradation can lead to transient species that may also represent a high pollution risk, which makes relevant the assessment of the main degradation mechanisms.

The photocatalytic degradation products of EE2 were also evaluated for photocatalysis process by GC-MS/MS. However, few by-products were identified, probably due to the interference of methanol that competes with the target hydroxylated by-products for the derivatization reagents.²⁹ Therefore, we consider appropriate to emphasize some mechanistic aspects of photocatalytic estrogen degradation discussed in the recent literature and the results obtained in this work, mainly to demonstrate the capability of photocatalysis to degrade estrogens with estrogenicity reduction.^{42,43}

According to the available information, estrogens can be degraded by two main oxidative mechanisms: abstraction of hydrogen by the photogenerated holes (h^+) and direct hydroxyl radical attack.⁴⁴ Moreover, according to Sun *et al.*,³⁴ semi-empirical simulations suggest that the C10 and C2 atoms in the phenol moiety of EE2 represent the most probable sites of attack by holes and hydroxyl radicals, respectively. Additionally, hydroxyl radicals can also attack the aliphatic ring,³² predominantly at the most reactive carbon atom in the α position with respect to the aromatic ring, i.e., C6.

In this work, substantial peaks of EE2 degradation products were observed to 5 and 15 min of reaction times, and after 30 min of treatment degradation products were not observed. During the first 5 min of photocatalytic treatment, only one by-product was observed (peak V, Figure 5A). According to Sun *et al.*,³⁴ EE2 can be directly oxidized by the photogenerated hole, abstracting an electron at the C10 atom and forming 17 α -ethynyl-hydroxy-1,4-estradien-3-one (EEO) via rearrangement of the phenol structure. This quinone derivative was also found by Frontistis *et al.*⁴⁵

In this study, it was not possible to identify the hydroxylated products 2-OH EE2 and 6-OH EE2 during the initial 15 min of heterogeneous photocatalysis. However, the GC-MS/MS analysis allowed the identification of further degradation products, as shown in Figure 5B. The four identified by-products (compounds **VI**, **VII**, **VIII** and **IX**) show dicarboxylic acid structure (muconic EE2), which characterized opening of the aromatic ring after addition of hydroxyl radicals at the C2 position. Further addition of hydroxyl radicals at the C6 position formed compound **IX** (6-OH), while compound **VIII** results from a subsequent conversion of the phenol moiety to a quinone-like moiety (6-oxo), as proposed by Sun *et al.*³⁴

Compounds **VI** and **VII** show a 4,5-dioxetane structure formed by reaction with superoxide radical anion, previously generated from the reduction of O_2 by e_{cb}^- . This intermediate may lead to the formation of muconaldehyde by further homolytic cleavage of the C–C bond.⁴⁶

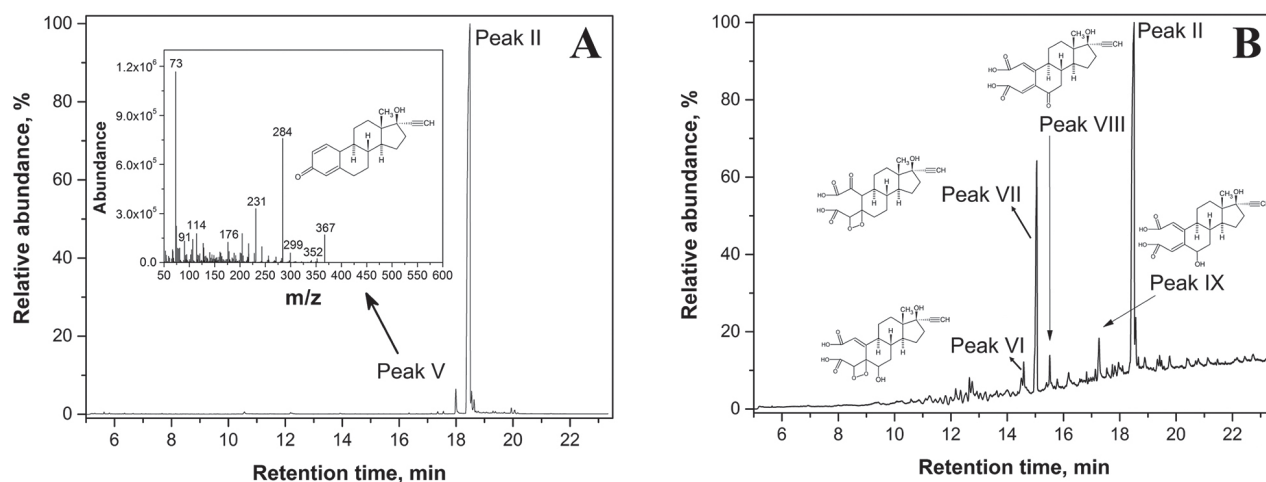


Figure 5. GC-MS analysis of by-products after UVA photocatalytic degradation of 17 α -ethinylestradiol (A) exposure time of 5 min; (B) exposure time of 15 min.

Although the reaction routes illustrated in Figure 6 represent the initial phase of the degradation process, they are important because, involving the degradation of the phenolic group of the molecule, entail a rapid withdrawal of estrogenicity, as confirmed by Ohko *et al.*⁴⁷ and Coleman *et al.*²¹ in studies involving recombinant yeast-based estrogen assay. Furthermore, comparing the results obtained in the present work with the literature, compounds **VI** to **IX** were determined unprecedentedly as derivatized compounds. It is important to point out the following mechanism considered the compounds without the mass of derivatizing agent and only the compounds in the squares were detected in the present work (the degradation products in dotted circles probably represent some intermediates of the compounds detected or another mechanism pathway).

Degradation of estrogens in spiked treated sewage samples

Finally, suspended and immobilized TiO₂ was used to study the degradation of estrogens in treated sewage samples. The sewage, with a chemical oxygen demand of less than 100 mg L⁻¹ and a pH of ca. 7, was spiked with 50 μ g L⁻¹ of E1, E2, and EE2 each and allowed to stand for 24 h under refrigeration. Subsequently, the samples were subjected to bench-scale treatment using suspended or supported TiO₂. After treatment, the samples were filtered through a glass fiber filter (0.6 μ m), preconcentrated by a factor of 100, and analyzed by HPLC-DAD-FLD.

The results (Figure 7) show that estrogens degradation by suspended TiO₂ is slow, achieving ca. 15 and 50% removal of E2 and EE2, respectively, after 60 min of reaction. These results are inferior to those obtained in aqueous solution (ca. 90%). Regarding immobilized TiO₂, the mean removal of both estrogens E2 and EE2

is approximately 85% after 60 min. These results are relatively close to the treatment of aqueous estrogens solutions at the same treatment time.

In case of suspended TiO₂, it is important to point out that after the first 15 min of treatment the catalyst forms aggregates that significantly reduce its contact surface. According to Malato *et al.*,⁴⁸ the size of the TiO₂ particles is directly affected by the pH, being smaller when the pH is furthest from the zero-charge pH. In these circumstances, the aggregation and cluster formation affect the ability of the suspension to absorb and transmit light. They also reported that in degradation processes started at a pH higher than the zero-charge point, which for TiO₂ is ca. 6.5, the pH tends to decrease during the treatment due to the formation of carboxylic acids before the mineralization process.⁴⁸ Thus, the variation observed in the estrogens concentration during TiO₂ suspensions process may be due to non-uniformity of the solution, caused by aggregation and sedimentation of the TiO₂. A similar trend was observed by Nasuhoglu *et al.*⁴⁹ in the treatment of levonorgestrel with suspended TiO₂.

The estrogens solubility may also influence the degradation system. EE2 is more soluble than E2; the latter probably is more retained in organic matter. As the photocatalytic degradation process occurs, it is easier to degrade EE2, which is more available in solution, than E2 that need to be released from the organic matter yet. During the suspended assays, possibly there was a competition for catalyst surface between all the constituents of wastewater samples and the estrogens.

The reduction of degradation efficiency related to wastewater treatment both for suspended and immobilized photocatalyst could also be due to the presence of hydroxyl radical scavengers, such as organic matter or inorganic

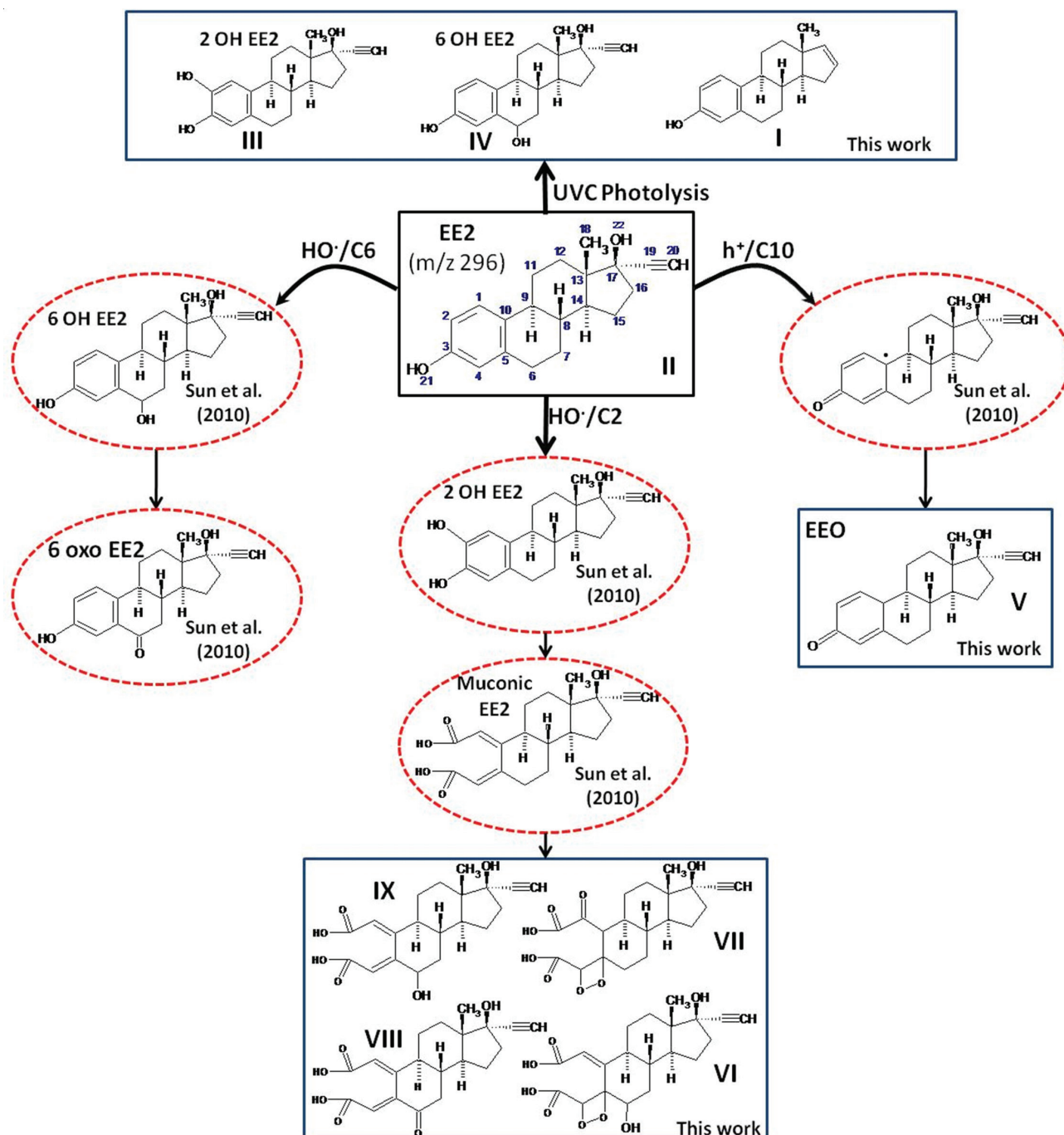


Figure 6. Illustration of the main reaction mechanism on the photo-assisted degradation of EE2. The dotted circles show the degradation products detected in the literature (adapted from reference 34) and the compounds in the squares were detected in the present work.

anions such as bicarbonate, chloride, and sulfate.⁵⁰ According to Frontistis *et al.*,⁵¹ the degradation of EE2 in wastewater samples needs reaction times three times longer than those needed for the treatment of aqueous solutions of estrogens, due to the presence of interfering matrix agents. Recently, Zhang *et al.*⁵² evaluated the influence of inorganic ions and dissolved organic matter on the removal of estrogenic activity from treated sewage samples submitted to TiO₂/UVA photocatalysis. The results

showed that ammonium phosphate causes a very significant negative effect because of its strong adsorption on the photocatalyst surface. Furthermore, adsorption of other substances on photocatalyst surface possibly competes with analyte adsorption and then reduce the number of active sites which are available to the estrogens degradation. In addition, the photon flux could be lower absorbed in such complex matrix, reducing the degradation efficiency.⁵³ The E1 degradation in wastewater samples could not

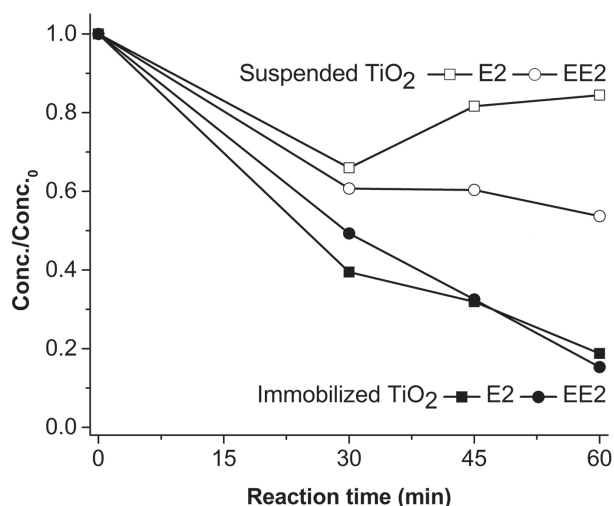


Figure 7. UVA bench photocatalytic degradation of E2 and EE2 by suspended and supported TiO₂ on spiked treated sewage samples (estrogens: 50 µg L⁻¹, pH: ca. 7.0).

be evaluated, due to strong matrix interference in the chromatographic analysis.

Conclusions

In this work, the high photosensitivity of the estrogens studied allowed its complete degradation by photolytic processes assisted by UVC radiation. For the artificial UVA-assisted process, suspended TiO₂ achieved degradations ca. 90% for the three estrogens in aqueous solution up to 60 min. However, when the same experimental setup was applied to estrogens degradation in wastewater samples, the photocatalytic process was not suitable and showed an expressive degradation rates decrease in the same time monitored (ca. 15% of E2 and 50% of EE2).

The photocatalytic performance of TiO₂ supported on glass rings allowed degradations of ca. 98% for all estrogens in aqueous solution, with negligible removal by adsorption, following exposure for 60 min. Surprisingly, the immobilized TiO₂ showed better estrogen removal (82% of E2 and 85% of EE2) when applied to degradation of analytes in wastewater samples. The TiO₂ immobilization possibly improve the estrogens degradation in such complex matrix since the influence on TiO₂ aggregation decreases.

The results for estrogens degradation in wastewater samples demonstrate the competition between the components of the medium and the analytes in the absorption of incident photons mainly for suspended TiO₂, as well as the need to extend the photocatalytic treatment to remove the analytes and the degradation products generated.

Studies involving the identification of degradation products by GC-MS evidenced rapid degradation of the estrogen molecule, particularly by reactions involving

the phenol moiety, which is responsible for the estrogenic activity observed in these compounds. Especially remarkable result was identifying the unprecedented hydroxylated degradation products from the treatment with TiO₂ in the first 15 min of reaction.

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