Effects of advanced oxidation processes (AOPs) on the toxicity of a mixture of pharmaceuticals


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Abstract The possibility of applying main AOP techniques, namely ozonation, H2O2/UV photolysis and TiO2 photocatalysis to provide a significant reduction of toxicity of pharmaceutical mixtures has been evaluated. For the preparation of the mixture six pharmaceuticals were chosen among those found at highest concentrations in Sewage Treatment Plant effluents, namely carbamazepine, clofibric acid, diclofenac, sulfamethoxazole, ofloxacin and propranolol.

The blue-green alga Synechococcus leopoliensis and the rotifer Brachionus calyciflorus were utilised to assess the toxicity of the mixtures after AOP treatments. All the toxicity tests were performed using chronic standardized bioassays. The best results were obtained with ozonation. With this type of treatment a complete removal of mixture toxicity on S. leopoliensis was obtained even after the shortest time of application (1 min). The ozonation treatment leads also to removal of all the pharmaceutical mixture toxicity on B. calyciflorus, by applying the oxidizing agent for at least for 2 minutes.

Keywords Advanced oxidation process; microalgae; pharmaceuticals; rotifers; toxicity

Introduction

Hundreds of tons of pharmaceuticals are annually released to the environment unmodified or as metabolites. It is worth noting that the most part of pharmaceuticals have been reported at trace levels (hundreds of nanograms per liter) also in the rivers and lake water thus indirectly confirming a certain capability to persist in surface waters. Conclusive data on adverse toxic effects on simple living organisms – at the low concentrations at which pharmaceutical molecules are found in the environment – are still lacking. However their xenobiotic nature and the precautionary principle would suggest to prevent their release to the environment by limiting the sources of pollution. It is nowadays generally accepted that the main sources are represented by Sewage Treatment Plant (STP) effluents and the manure from animal breeding (Kummerer, 2001; Heberer, 2002). A significant reduction of the inflow into the environment could be expected if already existing STPs would be up-graded by including tertiary treatments based on advanced oxidation processes (AOP) (Andreozzi et al., 2003a; Andreozzi et al., 2003b).

In the present work the investigations are concentrated on the removal from aqueous solutions of six pharmaceutical species (carbamazepine, diclofenac, clofibric acid, ofloxacin, sulfamethoxazole and propranolol) with special attention devoted to evaluate the effects of AOP treatments as ozonation, H2O2/UV and TiO2 photocatalysis on toxicity reduction.

Toxicity tests are carried out by exposing algae (Synechococcus leopoliensis) and invertebrates (Brachionus calyciflorus – rotifer) to aqueous samples containing the mixture of
six pharmaceuticals treated for different reaction times by means of the three oxidative techniques.

**Methods**

**Reagents**

Hydrogen peroxide (30% w/w, not stabilized) was purchased from Fluka. Carbamazepine [298-46-4], clofibric acid [832-09-7], diclofenac sodium salt [239-346-4], ofloxacin [82419-36-1], propranolol [3506-09-0] and sulfamethoxazole [723-46-6] were purchased from Sigma Aldrich Chemical Co.

**Oxidation experiments**

The solutions were prepared by dissolving six pharmaceutical species at the following concentrations: 7,070 µg l⁻¹ (carbamazepine), 325.5 µg l⁻¹ (propranolol), 11,200 µg l⁻¹ (clofibric acid), 2,800 µg l⁻¹ (diclofenac), 560 µg l⁻¹ (ofloxacin) and 2,240 µg l⁻¹ (sulfamethoxazole) in a moderately hard synthetic medium (MHW – EPA, 1994) constituted of NaHCO₃ 96 mg l⁻¹, CaSO₄·2H₂O 60 mg l⁻¹, MgSO₄ 60 mg l⁻¹ and KCl 4 mg l⁻¹. The pH was regulated at 7.6 by HClO₄ or NaOH.

The ozonation runs were carried out in a semicontinuously stirred tank Pyrex glass reactor (1.090 dm³), thermostated at 298 K. The apparatus used for the investigations has been previously described (Andreozzi et al., 1992). An ozonised oxygen stream generated by an ozone-generator (Fischer 502), with an ozone concentration of 0.42 mM, was fed at a flow rate of 36 dm³ h⁻¹ to the reactor containing the aqueous solution. The ozone concentration in the outlet gaseous stream was evaluated by continuous UV monitoring at 253 nm by means of a Varian UV spectrophotometer equipped with a quartz cell (optical length = 2.0·10⁻³ dm). Samples were withdrawn from the reactor after 1 minute (3.80 mg of ozone adsorbed in 0.8 dm³), 2 minutes (11.1 mg of ozone absorbed in 0.8 dm³) and 5 minutes (43.0 mg of ozone absorbed in 0.8 dm³) of reaction and ozone stripped by nitrogen bubbling.

The UV/H₂O₂ experiments were carried out at 298 K in an annular reactor equipped with a low-pressure lamp (by Helios Italquartz) with a monochromatic wavelength emission at 254 nm (Andreozzi et al., 1999). The radiation power (2.51·10⁻⁶ E s⁻¹) was measured by means of H₂O₂ actinometric measurements (Nicole et al., 1990). Two sets of experiments were performed with hydrogen peroxide starting concentrations of 5.0 and 10.0 mM. Residual hydrogen peroxide concentration was destroyed by adding catalase enzyme (1,300,000 U ml⁻¹ from Merck).

Samples were taken at different reaction times (1, 3 and 5 minutes) and rapidly analyzed by means of Hewlett Packard HPLC (HP 1100 L) equipped with a diode array detector and a Synergi 4u MAX-RP column. Two mobile phase solvents were used. Solvent A: 4 ml H₃PO₄ 85%, 50 ml methanol in 1 dm³ HPLC water, Solvent B was CH₃CN.

The flow rate was 1.0 mL min⁻¹, and the detection wavelengths were set at 200, 200, 210, 210 and 300 nm respectively for clofibric acid, sulfamethoxazole, diclofenac, carbamazepine, propranolol and ofloxacin.

The pH of aqueous solutions was measured by using Orion 960 pH-meter with a glass pH electrode.

The catalytic system for the photodegradation was prepared starting from titanium dioxide used free or immobilised, trying to always respect a concentration of about 0.3 g l⁻¹. Titanium dioxide was characterised by a surface area of 55 square meters per gram and it was supplied by Degussa. In the case of immobilisation PVC and polyaniline were used with titanium dioxide with a ratio between titanium dioxide and polyaniline of about 1.7 and between titanium dioxide and PVC of about 0.25. The source of light was a sun-light simulator (300 W) supplied by Panel Company, Cleveland, Ohio.
Toxicity tests with algae

The toxicity tests on the mixture of pharmaceuticals were carried out by using *Synechococcus leopoliensis* (Cyanophyta), strain UTEX 625, grown under the conditions previously described (Andreozzi et al., 2002). The toxicity tests were performed as in Ferrari et al. (2003). All the experiments were conducted in triplicate and repeated three times.

Toxicity tests with invertebrates

The Rotifer 2-d test procedure (Rotoxkit™) measures toxicity on the reproduction of the rotifer *Brachionus calyciflorus* after 48 hours of exposure. It was conducted according to the standard AFNOR T90-377 (2000). Test animals were obtained by hatching cysts of *B. calyciflorus*, supplied by MicroBioTests Inc. (Fazantenpark 9, 9800 Deinze, Belgium). Cyst hatching was initiated in MHW about 20 h before the start of the test at 25 ± 1°C in a temperature-controlled chamber under a light intensity of 3000 lux. After 18 h of incubation, cysts were checked to ensure that test animals were collected within 2–3 h of hatching. The assay was carried out in a 48-well microplate, which allowed testing of five samples, or dilutions of them, and one control, with eight replicates. Nine hundred microlitres of the tested pharmaceutical dilution (MHW for the negative control) was introduced in each well and then completed with 100 µl of a fresh suspension of green algae *Pseudokirchneriella subcapitata*. Algae were diluted to ensure a final concentration of 106 cells/ml in each well. The test was initiated by transferring one newly hatched rotifer into each well. Next, the microplate was covered to reduce evaporation and then incubated at 25 ± 1°C in the temperature-controlled chamber in darkness. After 48 h of incubation, the total number of living females per well was counted.

Data analysis

To investigate the capability of AOP techniques to reduce the toxicity of the pharmaceutical mixture, two types of sample are produced for each treatment tested: the mixture non-submitted to treatment, corresponding to the positive toxicity control, and the treated mixture, using various oxidative conditions. For algae and invertebrate bioassays, the toxicity removal was determined by hypothesis tests, comparing the data obtained from treated pharmaceutical mixture to negative control (MHW medium) or positive control. Student t-tests, after verifying the Shapiro-Wilks’ test for normality and the Hartley’s test for homogeneity of variance, were used. Calculations were performed using TOXSTAT 3.0 software (Gulley et al., 1989).

Results and discussion

In Figure 1 the removal percentages of six pharmaceuticals recorded in the experiments with ozone are reported. It is clear that for all the components except clofibric acid a complete removal was observed after 2 minutes of treatment. Similar results were obtained with H₂O₂/UV system (data not shown). TiO₂ photocatalytic experiments were performed for 48 h for suspended TiO₂ and membrane immobilized TiO₂ mode since for this reaction time removals higher than 80% were achieved for each pharmaceutical (data not shown). The results on the investigated mixtures indicate for both types of experiment an incomplete removal of six pharmaceuticals.

Toxicity tests with algae

Based on the data obtained from preliminary experiments on concentration-response analyses, a concentration of the mixture causing 80% growth inhibition of *S. leopoliensis* was selected to assess the efficiency of different types of treatment on the removal of the mixture toxicity (Table 1).
Both UV/H₂O₂ and ozonation afforded the complete removal of the toxicity, even after 1 minute of treatment. Longer treatments (3 and 5 minutes for UV/H₂O₂ and 2 and 5 minutes for ozonation) did not lead to an increasing toxicity, excluding the possibility that toxic products could be generated during the treatments. On the other hand, a stimulation of the algal growth was observed with both treatments at times longer than 1 min. The growth stimulation was generally not relevant (less than 10%), with the exception of 5 min treatment with H₂O₂/UV, which caused about 40% increase of the growth.

Toxicity tests with invertebrates
Normal reproduction of B. calyciflorus in MHW medium (negative control) presents a large variability, with a standard deviation up to 15%. On the other hand, the toxicity of the non-treated pharmaceutical mixture (positive control) is reproducible between each bioassay; this mixture induces 80 ± 7% of inhibition of B. calyciflorus reproduction. These values, calculated from all bioassay data, are used as negative and positive references for the statistics tests, and are reported in Table 2.
After 1 min of ozonation the toxicity of the mixture is reduced to 50% but with still an effect on rotifer reproduction (45% of inhibition). The total removal of toxicity occurs after 2 min of treatment. For H₂O₂/UV treatment, it was preliminarily checked that the addition of catalase to destroy the remaining H₂O₂ does not result in any additional toxic effects. Using 5 mM of H₂O₂, the UV exposure has to be applied at least for 5 min to provoke no toxicity effect. The same result is observed with 10 mM H₂O₂, whatever the UV exposure duration, respectively 3 and 5 min. The treatment using UV during 48 h and TiO₂ in suspension in the medium induces a slight reduction of the toxicity of the pharmaceutical mixture (9%). Moreover, when TiO₂ is applied on a membrane and submitted to UV during 48 h, there is no effect on the total toxicity of the mixture.

### Table 2 Effects of pharmaceutical mixture on rotifer reproduction submitted to ozonation, H₂O₂/UV and TiO₂ photocatalytic treatment, under various conditions

<table>
<thead>
<tr>
<th>Treatment Conditions</th>
<th>time</th>
<th>% effect (±SD)</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>80.2 ± 6.7</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative control</td>
<td>0 ± 14.1</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>Ozonation</strong></td>
<td></td>
<td></td>
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<tr>
<td>1 min</td>
<td>45.1 ± 19.3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2 min</td>
<td>−2.5 ± 27.4</td>
<td>&lt;0.001</td>
<td>0.422</td>
</tr>
<tr>
<td>5 min</td>
<td>2.5 ± 29.1</td>
<td>&lt;0.001</td>
<td>0.949</td>
</tr>
<tr>
<td><strong>H₂O₂/UV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[H₂O₂] = 5 mM</td>
<td>0 min 87.3 ± 6.1</td>
<td>0.250</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>3 min 25.3 ± 13.5</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>5 min 5.8 ± 20</td>
<td>&lt;0.001</td>
<td>0.537</td>
</tr>
<tr>
<td>[H₂O₂] = 10 mM</td>
<td>0 min 82.2 ± 9.5</td>
<td>0.881</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>3 min 3.4 ± 14.7</td>
<td>&lt;0.001</td>
<td>0.233</td>
</tr>
<tr>
<td></td>
<td>5 min 6.7 ± 21.4</td>
<td>&lt;0.001</td>
<td>0.277</td>
</tr>
<tr>
<td><strong>TiO₂/Photocatalysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TiO₂ suspended</td>
<td>48 h 74.6 ± 16.3</td>
<td>0.017</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TiO₂ membrane</td>
<td>48 h 84.7 ± 14.5</td>
<td>0.300</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Conclusions

Ozonation and H₂O₂/UV appear to represent promising techniques for the treatment of pharmaceutical mixtures. They largely elicit the toxicity of these compounds on rotifers after respectively 2 and 3 minutes of treatment. On the other hand, shorter treatments (1 min) seem to be the optimal condition for algae, whose growth was stimulated when ozonation or H₂O₂/UV were applied for 3–5 minutes. This could be due to the presence of intermediates originating from the degradation of pharmaceuticals, which are used as a carbon source by the alga. Poor results are obtained by means of the TiO₂ photocatalytic system, probably due to the formation of toxic oxidation intermediates. These results suggest that ozonation and H₂O₂/UV are capable of reducing the toxicity due to pharmaceutical species. An extension of the investigations to the treatment of real STP effluents is thus required to assess if these processes could be successfully applied even in the presence of more complex aqueous matrices.

Acknowledgements

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