Pharmaceuticals degradation by UV and UV/H$_2$O$_2$ treatments

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Abstract The degradation by UV and UV/H$_2$O$_2$ treatments of the pharmaceutical intermediate 5-methyl-1,3,4-thiadiazole-2-methylthiol (MMTD-Me) has been investigated and compared to that of its parent compound [5-methyl-1,3,4-thiadiazole-2-thiol (MMTD)] previously studied. The investigation has been carried out with a 17 W low pressure mercury lamp, at room temperature, with an initial MMTD-Me concentration of 1 mg/l and with a molar ratio H$_2$O$_2$/substrate of 100/1. The results show that: (i) the complete MMTD-Me removal is achieved within 60 and 20 minutes by UV and UV/H$_2$O$_2$ treatment respectively; (ii) the UV only irradiation does not cause any MMTD-Me mineralization; (iii) the UV/H$_2$O$_2$ treatment, after 4 hours, leads to a complete mineralization of MMTD-Me organic sulfur and to a partial mineralization of carbon and nitrogen (79 and 16% respectively). Degradation by-products identification, performed by HPLC-UV-MS, revealed that the UV only irradiation gives rise to the sequential transformation of MMTD-Me into two by-products one of which, the last one, accumulates in the solution. Conversely, the UV/H$_2$O$_2$ treatment leads to the formation of two intermediate by-products that undergo further degradation with the breakdown of the thiadiazole ring. These results confirm the effectiveness of UV based processes, alone or in combination with H$_2$O$_2$, in degrading pharmaceutical intermediates.

Keywords Chemical oxidation; degradation by-products; pharmaceutical intermediates; UV; UV/H$_2$O$_2$

Introduction

The industrial production of pharmaceuticals is often based on low yield chemical reactions resulting in incomplete conversion of intermediates. In such cases, after the synthesized pharmaceuticals have been recovered, the excess intermediates are discharged with wastewater and frequently are not effectively removed by conventional treatments. This represents a potential threat for the environment and justifies the current interest for assessing the effectiveness of advanced treatments to remove such intermediates.

In the past decade photolytic processes have been proposed as a reliable option for the treatment of organic pollutants in drinking and waste water because of their effectiveness to degrade a wide range of contaminants (Huang et al., 1993). Attractive features of photolytic treatments are: (i) the progressive decrease of treatments costs due to the introduction of relatively cheap, low energy UV lamps; (ii) avoidance the scaling of the lamps using “non-contact” reactors; (iii) simultaneously using UV rays and chemicals [i.e., ozone (O$_3$) or hydrogen peroxide [H$_2$O$_2$] for producing the strongest oxidant species after fluorine, i.e. °OH radicals; (iv) the widespread perception that UV-based processes are “environmentally-friendly” technologies because of the low toxicity, if any, of the treated effluents. UV-based treatments are particularly effective for removing persistent organic pollutants from water and/or wastewater but have specific technological requirements for the wastewater such as by appropriate chemical composition (e.g., some constituents in the water could absorb UV light and hence decrease the effect on target pollutants) and low content of suspended solids (scattering of UV light must be avoided).

From the chemical standpoint, UV degradation of organics usually takes place through photooxidation reactions. These reactions imply the excitation of the considered
compounds by UV light followed by the homolysis of a covalent bond that gives rise to an organic radical which could further react through a radical mechanism and/or an electronic transfer to ground-state molecular oxygen that leads to the formation of a radical cation (Legrini et al., 1993). An important parameter that affects the rates of both routes is the excitation wavelength. Low-pressure lamps, having irradiation at 253.7 nm, beside their good efficiency for water disinfection (EPA, 1996) have shown usefulness for the degradation of substituted aromatics. However, they are not effective for chlorinated aliphatics removal (Sundstrom et al., 1986). By contrast, medium-pressure lamps, having a broad emission band ranging from 210 to 230 nm, have shown higher efficiency for removing chlorinated organics through the homolysis of the C-Cl bonds (Toy et al., 1990).

The process performances is, in term of organic degradation rate, more effective when UV treatment is carried out in the presence of H2O2. This is due to the photolysis of H2O2 into the short-lived •OH radical (H2O2 + hv → 2 •OH) able to degrade organic compounds by hydrogen abstraction and/or electron transfer and/or electrophilic addition reactions. The rate of photolysis of aqueous H2O2 is pH dependent because of the greater molar absorption coefficient of the peroxide anion (HO2−) at 253.7 nm (Nicole et al., 1990). Although organics removal by UV and UV/H2O2 is often achieved successfully, the complete destruction of these contaminants (i.e. the total mineralization of organic carbon to carbon dioxide) is seldom obtained. Accordingly, the formation of several by-products is usually expected (Millet et al., 1998) and their identification is a key step for assessing the health risk associated with UV based treatments (Ho and Bolton, 1998).

In the present paper, the focus was on the effectiveness of UV and UV/H2O2 treatments to remove and/or degrade the pharmaceutical intermediate 5-methyl-1,3,4-thiadiazole-2-methylthio (MMTD-Me). The results, in terms of removal effectiveness, photochemical behavior and by-products formation, have been compared to that of its parent compound 5-methyl-1,3,4-thiadiazole-2-thiol (MMTD) previously investigated (Lopez et al., in press). The chemical structures of MMTD-Me and MMTD are both shown in Figure 1.

Both compounds were detected during a groundwater quality survey carried out in northern Italy (Guardini, 1999). In particular, MMTD is a pharmaceutical intermediate used for the synthesis of cefazolin, a cephalosporin antibiotic (Won et al., 1998). Moreover, MMTD has been shown to be potentially harmful because it may play an important role in the pathophysiology of hypoprothrombinemia (Kerremans et al., 1985). MMTD-Me is a metabolite of MMTD usually formed during its biological degradation (Guardini, 1999).

### Experimental

**Reagents and Chemicals.** 5-methyl-1,3,4-thiadiazole-2-thiol (MMTD) was obtained from Aldrich (Milwaukee, WI, USA) and used as received (99% purity), 5-methyl-1,3,4-thiadiazole-2-methylthio (MMTD-Me) was synthesized according to usual procedure for –SH group derivatization (Oae, 1977). H2O2 (30% w/w) was from J.T.Baker (Baker, Gross-Gerau, Germany). HPLC grade solvents (water and methanol) were purchased from Fluka (Sigma-Aldrich, Milwaukee, WI, USA). High purity water from a Milli Q-Water System.
(Millipore, Bedford, MA, USA) was used for preparing all aqueous solutions. Potassium peroxodisulfate and uridine were obtained from Baker and Aldrich respectively.

**Photochemical experiments.** Experiments were carried out at room temperature in a 500 ml cylindrical Pyrex reactor. A 17 W low-pressure mercury lamp, obtained from Helios Italquartz (Milan, Italy), emitting at 185 and 254 nm was used. The lamp was introduced into the reactor and kept separated from the aqueous solution by a quartz cooling jacket that does not transmit significant amount of 185 nm radiation. In this configuration the light path was 1.9 cm. The solution was stirred by a magnetic bar throughout the duration of the experiment in order to keep the solution homogeneous. Before each experiment, the lamp was warmed up for at least 15 min to ensure stable lamp-output. The lamp was calibrated by actinometry experiments using potassium peroxodisulfate and uridine following procedures reported elsewhere (Mark et al., 1990; von Sonntag and Schuchmann, 1992). From such experiments the average incident photonic flux was $2.8 \times 10^{-6}$ einstein·s$^{-1}$ at 20 °C which corresponds to a power output (fluence) of 48 W·m$^{-2}$ and an effective reactor light path of 1.85 cm. MMTD and MMTD-Me quantum yield was calculated by means of an usual procedure (Nick et al., 1992). In a typical photochemical experiment, 500 ml of substrate aqueous solution were put in the reactor and, for UV/H$_2$O$_2$ experiments, the appropriate amount of H$_2$O$_2$ was added in order to have a molar ratio H$_2$O$_2$/substrate of 100/1. Afterwards, the UV-lamp was introduced into the reactor and at each fixed time a 5 ml sample was taken and immediately analyzed by HPLC.

**Analytical determinations.** UV measurements were performed with a Cary 1E UV-Vis spectrophotometer (Varian Inc., Palo Alto, CA, USA). The concentration of unreacted MMTD was monitored by HPLC-UV with a 1050-Ti chromatographic system (Agilent Technologies, Palo Alto, CA, USA) equipped with a Chromsphere 5B 5 µm, 250 × 3 mm column, a 10 × 2 mm pre-column, both from Chrompack (Walnut Creek, CA, USA) and a 1050 series variable wavelength detector set at 285 nm. Samples, injected by a Gilson 234 autosampler (Gilson, Middleton, WI, USA) equipped with a 9010 Rheodyne valve and a 50 µl loop, were eluted by a water/methanol 70/30 mixture at 0.6 ml/min. The detection limit was about 0.005 mg/l and reproducibility was within ±5%. By-products identification was performed by HPLC-UV-MS using the above reported HPLC system interfaced to an API 165 mass spectrometer (Applied Biosystems/MSD Sciex, Foster City, CA, USA) equipped with a turboionspray interface. The interface conditions were: nebulizer gas (air) = 1.2 l/min, curtain gas (nitrogen) = 1 l/min, turboionspray gas (nitrogen at 300°C) = 6 l/min, needle voltage = +5200 or −4500 V, orifice voltage = +30 or −25 V and ring voltage = +160 or −200 V for positive and negative ions analysis respectively. Samples, injected by the autosampler, were analyzed by running a gradient, from 10/10/80 (1% acetic acid in methanol/methanol/water) to 10/70/20 in 10 min, which was maintained for 3 min. The flow from the HPLC-UV, set at 254 nm, was split to allow 200 µl/min to enter the turboionspray interface. Total organic carbon (TOC) analysis were carried out by a TOC-5050 analyzer (Shimadzu, Tokyo, Japan) equipped with a Platinum catalyst on alumina spherical support. The quantitative determination of nitrite, nitrate and sulfate were carried out by ion chromatography using a Dionex 4000i LC system (Dionex, Sunnyvale, CA, USA) equipped with a ASRS-ultra suppressor, operating in recycled mode, and a conductivity detector. Samples, injected via a 50 µl loop, were eluted at a flow rate of 1 ml/min through an analytical column (IonPac AS-14 250 × 4 mm) equipped with a pre-column (IonPac AG-14 50 × 4 mm) by an aqueous buffer solution of NaHCO$_3$/Na$_2$CO$_3$ 1.0 mM/3.5 mM.
Results and discussion

As shown below in figures 1 and 2, significant removals were measured for MMTD-Me and MMTD during both UV and UV/H\textsubscript{2}O\textsubscript{2} treatments. However, using only H\textsubscript{2}O\textsubscript{2}, no removal was measured for either compound. The decays recorded for MMTD and MMTD-Me during UV treatment are shown in Figure 2. This figure shows that both compounds are UV degraded albeit with different rates. More than 60 and 45 min are necessary to completely remove MMTD and MMTD-Me respectively. Such a difference in rates can be only due to different values of quantum yield of photolysis and molar extinction coefficient for the two compounds.

The above conclusion was confirmed measuring the molar extinction coefficients of the two compounds by UV spectroscopy (2100 and 4970 M\textsuperscript{-1}·cm\textsuperscript{-1} for MMTD and MMTD-Me respectively) and calculating the quantum yield values from the data of figure 2 (12.0 and 14.1 mmol·einstein\textsuperscript{-1} for MMTD and MMTD-Me respectively). From these values it has been also possible to calculate the extent (%) of MMTD and MMTD-Me removal in the case of an UV dose of 250 J·m\textsuperscript{-2}, i.e. the dose commonly used to disinfect drinking water. The results gave an extent of less than 1% confirming the finding of Nick et al. (Nick et al., 1992) that UV disinfection and UV degradation of chemicals operate on very different fluence scales.

As shown in figure 3, where the decays of the investigated compounds during the UV/H\textsubscript{2}O\textsubscript{2} treatment are depicted, the presence of H\textsubscript{2}O\textsubscript{2} greatly speeds up the degradation rates compared to UV only treatment shown in figure 2. In fact, MMTD and MMTD-Me are completely removed within 10 and 20 min respectively. Such a “speed up” effect is due to the photolysis of H\textsubscript{2}O\textsubscript{2} that gives rise to the formation of hydroxy radicals (H\textsubscript{2}O\textsubscript{2} + h\nu \rightarrow 2\cdotOH). These radicals, that can be generated by several methods (Huang et al., 1993), are known to react with organics at high rates and with poor selectivity (Haag and Yao, 1992).

Comparing figures 2 and 3 it is also evident that MMTD, i.e. the compound removed at the slowest rate during UV treatment, is degraded at the fastest rate during the UV/H\textsubscript{2}O\textsubscript{2} treatment. Considering the similar chemical structure of the two compounds, this result is a consequence of the different rate of the \cdotOH attack to the –SH and –SCH\textsubscript{3} groups. The former group, in fact, is more electrons rich and consequently reacts faster with \cdotOH that is an electrophilic species.

Taking into account that organics degradation by \cdotOH (UV/H\textsubscript{2}O\textsubscript{2}) is expected to cause the mineralization of treated substrate, some parameters related to mineralization (TOC, SO\textsubscript{4}\textsuperscript{2\textendash}, NO\textsubscript{3}\textsuperscript{\textendash}) were monitored during the reaction. The results depicted in Figure 4 indicate that to the fast MMTD and MMTD-Me disappearance shown in Figure 2 corresponds a slow mineralization. Referring to MMTD, after four hours, an almost stoichiometric
formation of $\text{SO}_4^{\text{2-}}$ was recorded whereas the extent of carbon and nitrogen mineralization was only 59% and 14% respectively. Similar results were obtained for MMTD-Me with the $\text{SO}_4^{\text{2-}}$ formation stoichiometric and the carbon and nitrogen mineralization extent at 79% and 16% respectively. Such significant mineralization degrees obtained during UV/H$_2$O$_2$ treatment are the result of the high reactivity of the $\cdot$OH radicals formed during the UV photolysis of H$_2$O$_2$. The UV photolysis of both compounds in absence of H$_2$O$_2$, did not produce any measurable mineralization of carbon, nitrogen and sulfur but only the formation of elemental sulfur.

Based on the above results, it is evident that MMTD and MMTD-Me disappearance during both treatments should be accompanied by the formation of some by-products. In fact, the organic carbon mineralization in any case is only partial. Furthermore, the slow mineralization of organic carbon, sulfur and nitrogen observed during the UV/H$_2$O$_2$ treatment after the complete removal of both compounds has been achieved, suggests that the by-products initially formed are degraded later at rates slower than the original compounds. The identification of such degradation by-products was performed by HPLC-MS as described in the experimental section. In Table 1 are reported for each identified by-product the molecular weight (MW), the main fragment ion masses observed in their mass spectra and the treatment in which it was identified. Looking at Table 1 it is possible to note that during the MMTD degradation by the UV alone, only by-product 1 was identified. Its MW, lower than that of MMTD, is consistent with the MMTD chemical structure without the atom of sulfur of the thio group (-SH). This by-product was also identified, together with the MMTD molecule, during the MMTD-Me degradation by the UV only.

Table 1 also show that MMTD degradation by UV/H$_2$O$_2$ gives rise to the formation of by-products 2-7. The MW of by-product 2 is consistent with the sulfinyl derivative of by-product 1. The proposed structures of by-products 1 and 2 also justify their mass spectrometric detection only in positive ion. By-products 3-7 have MW greater than that of MMTD suggesting that coupling and oxidation reactions have occurred. In particular, the MW of by-products 3 and 4 are consistent with the MMTD chemical structure in which the –SH group has been oxidized to –SO$_3$H and –OSO$_3$H respectively. These groups make the molecules strongly acid and render them mass spectrometrically detectable only in negative ion. By-products 5-7 have MW corresponding to chemical structures resulting or from coupling reactions [between two MMTD molecules (by-product 5); between one MMTD and one by-product 1 molecule (by-product 6)] or from consecutive oxidations of the by-product 6 (by-product 7). These structures were also confirmed by the interpretation of the relative mass fragment ions reported in table 1. In fact, the m/z 164 and 132 ions of
by-product 5, correspondent to the loss of the 5-methyl-1,3,4-thiadiazole and MMTD ions respectively, suggest the presence of the disulfide group. As for the by-product 6 only the m/z 132 ion, correspondent to the loss of the 2-methyl-1,3,4-thiadiazole, was found. This indicates the presence of a single sulfur atom that bridges the two thiadiazole rings being the m/z 155 and 190 ions correspondent to the thiadiazole rupture (loss of CH$_3$-CS-NH$_2$ and CH$_3$CN respectively). As for by-product 7, the m/z 231 and 164 ions, correspondent to the loss of four oxygen atoms and to the sulfonyl derivative of MMTD respectively, indicate that the four oxygen atoms are located on the two thiadiazole rings. Moreover, the proposed structures of by-product 5-7, having proton acceptor sites (sulfur and nitrogen atoms), also justify their only mass spectrometric detection in positive ion. The MMTD-Me degradation by UV/H$_2$O$_2$ leads to the formation of by-product 1 and 8 (Table 1). By-product 8 has a MW 16 amu greater than that of the parent compound suggesting a sulfinyl derivative of MMTD-Me structure in which one of the two sulfur atoms has been oxidized.

It is important to point out that MMTD degradation by UV only causes its slow transformation into the by-product 1 which accumulates into the solution together with elemental colloidal sulfur that makes turbid the solution. As for MMTD-Me, a similar behavior was obtained with the sole difference that by-product 1 was formed through the formation of MMTD as intermediate. Referring to the degradations by UV/H$_2$O$_2$, MMTD and MMTD-Me by-products were formed almost at the same time and then completely degraded within 3 hours. At this time, no by-products were detected by HPLC-MS. However, as most of the organic nitrogen was not mineralized (see figure 4), it is presumable that the organic carbon left in the solution is present as polar low molecular weight nitrogen-containing

**Table 1** Proposed chemical structures, [M-H]$^-\$ or [M+H]$^+$ ions and fragmentation masses of the identified by-products (for experimental conditions see the text).

<table>
<thead>
<tr>
<th>By-product</th>
<th>Treatment in which the by-product was identified</th>
<th>Proposed structure</th>
<th>MW</th>
<th>[M+Na]$^+$, [M+H]$^+$ and fragment ion masses (relative intensity)</th>
<th>[M-H]$^-$ and fragment ion masses (relative intensity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMTD-Me</td>
<td></td>
<td></td>
<td>146</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(MMTD-Me)+UV</td>
<td></td>
<td>132</td>
<td>131(100), 90(13)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(MMTD)+UV/H$_2$O$_2$</td>
<td></td>
<td>116</td>
<td>117(52), 99(40), 87(100)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>(MMTD)+UV/H$_2$O$_2$</td>
<td></td>
<td>180</td>
<td>179(100), 106(15), 58(30)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>(MMTD)+UV/H$_2$O$_2$</td>
<td></td>
<td>196</td>
<td>195(18), 58(100)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>(MMTD)+UV/H$_2$O$_2$</td>
<td></td>
<td>262</td>
<td>285(10), 263(100), 164(11), 158(10), 132(23), 59(7)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>(MMTD)+UV/H$_2$O$_2$</td>
<td></td>
<td>230</td>
<td>253(14), 231(56), 190(19), 155(100), 132(18), 99(7), 59(23)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>(MMTD)+UV/H$_2$O$_2$</td>
<td></td>
<td>294</td>
<td>317(30), 295(92), 231(99), 164(100)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>(MMTD-Me)+UV/H$_2$O$_2$</td>
<td></td>
<td>162</td>
<td>163(100), 148(53), 117(17), 101(98)</td>
<td></td>
</tr>
</tbody>
</table>
by-products. These compounds, because of their low molecular weight and high polarity, cannot be retained by the used HPLC column and, accordingly, were not detected nor identified.

**Conclusions**

A laboratory investigation has been carried out to investigate the degradation of the pharmaceutical intermediate MMTD-Me by UV and UV/H$_2$O$_2$ in aqueous solution and its behavior compared to that of MMTD previously investigated. The main results have been the following:

- using only H$_2$O$_2$ no degradation and/or removal took place for either of the two compounds;
- with the UV alone treatment more than 60 and 45 min were necessary to completely remove MMTD and MMTD-Me respectively at an initial substrate concentration of 1 mg/l;
- the UV/H$_2$O$_2$ treatment completely degrades MMTD and MMTD-Me within 10 and 20 min respectively;
- the UV alone treatment, gives rise to the MMTD and MMTD-Me transformation into a single end by-product, the same for both, that continuously accumulates in solution together with elemental sulfur;
- by contrast, in the presence of H$_2$O$_2$, UV irradiation of both investigated compounds leads to an almost complete mineralization of the sulfur and to a partial mineralization of the carbon and nitrogen;
- for both compounds, the UV/H$_2$O$_2$ treatment leads to the formation of several by-products that undergo further degradations with the rupture of the thiadiazole ring.

These results confirm the effectiveness of UV based processes, alone or in combination with H$_2$O$_2$, in degrading pharmaceutical intermediates potentially harmful for the environment.

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**References**


