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Ozonation of carbamazepine, diclofenac, sulfamethoxazole and trimethoprim and formation of major oxidation products

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ABSTRACT

The degradation of four pharmaceuticals, carbamazepine (CBZ), diclofenac (DCF), sulfamethoxazole (SMX) and trimethoprim (TMP) by ozonation was studied under a range of experimental conditions, including ozone dosage and concentration of target compounds. The concentration profile of the pharmaceuticals and detection of any by-products formed was carried out using liquid chromatography mass spectrometry. CBZ, DCF, TMP and SMX at initial concentration of 5 mg/L each were degraded to below the method detection limit (1 μg/L) when they reacted with 1.6, 2.3, 2.8 and 4.5 mg/L of ozone, respectively. For each parent compound several by-products were detected after the ozone treatment. A number of these by-products have not been previously reported in the literature. Some of these by-products were founds to be quite resistant to ozone up to applied ozone dosages of 15 mg/L.

Keywords: Ozonation; By-product identification; Pharmaceuticals; Wastewater treatment

1. Introduction

There is a great deal of interest in the occurrence and fate of pharmaceutically active compounds (PhACs) in wastewater and sewage treatment [1]. Classes of pharmaceuticals include antipyretics, blood lipid regulators, analgesics, antidepressants, antibiotics, chemotherapy agents and contraceptive drugs. After administration, they are partially metabolised and excreted in the urine and/or faeces, and consequently enter into sewer. It is well established that many of PhACs cannot be fully removed during conventional sewage [1–6]. Therefore, many PhACs have been detected in a number of sewage treatment plant effluents and also in surface water and groundwater in numerous countries [3,7–9].

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The frequent occurrence of PhACs in the aquatic environment and sometimes even in drinking water has raised significant concerns regarding their possible effects on ecological and public health. Potential negative impacts caused by PhACs include aquatic toxicity, antibiotic resistant development and endocrine disruption [2,10–12]. For example, the occurrence of antibiotics such as sulfamethoxazole (SMX) and trimethoprim (TMP) in the environment is directly associated with the resistance to antibiotic showed by bacteria in natural media [13]. Diclofenac (DCF) residues were found to have had an environmental influence, causing a decrease in vulture population in Pakistan [14]. It has been reported that, the lowest observed impact for cytopathology in the livers, kidneys and gills of rainbow trout occurred at concentration of 1 μg/L [15]. Carbamazepine (CBZ) has been considered as toxic to aquatic organism including bacteria, invertebrates, algae and fish [16,17]. However, further research is required to investigate the possible chronic health impacts related to long-term exposure of PhACs to humans and the environment [18,19]. Thus, the need to effectively remove PhACs from wastewater has driven the applications of advanced treatment processes such as membrane bioreactor, reverse osmosis and ozonation [20–22].

Ozonation is known to be an efficient treatment for the destruction of PhACs. A key feature of ozone is its decomposition into hydroxyl radicals (·OH) which is one of the strongest oxidants in water [23–27]. Disinfection takes place mainly through O_3, whereas oxidation processes might occur via both ozone and OH radicals [28]. As a result, disinfection and oxidation can be achieved simultaneously if ozone reactions are responsible for the oxidation. Ozone is a very selective electrophile which reacts directly with double bonds, activated aromatic structures, or heteroatoms (e.g. sulphur and nitrogen), which are dominant substituent groups of numerous drugs, including DCF, SMX and CBZ. Alternatively, drugs without reactive sites, such as clofibric acid, bezafibrate and iopromide are more amenable to hydroxyl radicals, which react less selectively and at higher molecular rates [29,30]. Consequently, most PhACs can be effectively removed by ozonation.

A challenge associated with ozone treatment of PhACs is the formation of by-products. It has been well documented that the oxidation of several PhACs by ozone can lead to the formation of by-products [31–35]. Such by-products could potentially have adverse impacts on aquatic life and human health, and may in fact be more recalcitrant or toxic than their parent compounds [36,37]. Therefore, analytical techniques are advantageous in order to determine the removal of the target compounds and identify the formation of any new products.

The main objective of the current work was to investigate the degradation of four PhACs by ozone. Furthermore, liquid chromatography–mass spectrometry (LC-MS) methods were used to detect any major oxidation products formed during the treatment. Simple aqueous model systems were employed consisting of only the target pharmaceutical at a concentration sufficient to enable detection of the by-products. This approach has been successfully used in previous studies in order to identify by-products [29,38–43]. This initial study was mainly focussed on investigating the rate of degradation of the four (parent) pharmaceuticals and the evolution of major by-products, with particular emphasis on finding compounds not reported previously in the literature. Whilst the absolute rates of reactions are concentration dependent, these model systems do give useful information about the relative stability of the pharmaceutical compounds with ozone, and the persistence of some of the by-products, of relevance to wastewater treatment conditions. Mass spectrometric data of the detected compounds was primarily used here to confirm findings from previous studies.

2. Materials and methods

2.1. Materials and chemicals

Four pharmaceuticals, namely CBZ, DCF, SMX and TMP, were selected for investigation (Fig. 1). They were of analytical grade and were purchased from Sigma–Aldrich (Sydney, Australia). All other chemicals and solvents used in this study were of analytical or high-performance liquid chromatography (HPLC) grade and supplied by VWR (Sydney, Australia). Milli-Q quality water was used in all experiments.

2.2. Ozonation system and protocol

Ozone experiments were carried out using an ozone generator (CD10/AD, Clear Water Tech, USA), a gas phase ozone concentration analyser (ME820, Ebara Jitsugyo, Japan) together with a gas flow meter, and a 1-L glass ozone reactor vessel. The ozone generator was capable of producing up to 1.3 g/h of ozone using instrumental grade air as the oxygen source. The amount of ozone used in the reactor was determined by mass balance from the amount generated and the amount of ozone in the gas stream post-reaction. A detailed description of this ozonation system is available elsewhere [44].

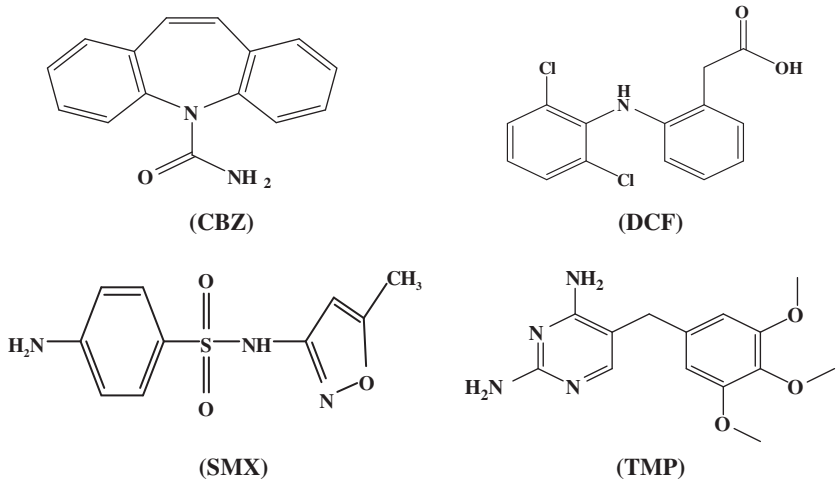

Fig. 1. Molecular structures of the four selected pharmaceuticals.

2.3. Experimental protocol

Stock solutions of CBZ, DCF, SMX and TMP were prepared with a concentration of 5 g/L in pure methanol. These solutions were stored in amber bottles and kept in a freezer at −18˚C prior to use. A series of standard solutions were prepared for the calibration at 1, 10, 50, 100 and 200 μg/L of each analyte.

For the experiments, 5 mg/L of each pharmaceutical were prepared in Milli-Q water and directly transferred into the ozone reactor. Relatively high concentrations of target pharmaceuticals (5 mg/L) compared with those found in wastewater samples were used in order to produce the expected by-products at sufficient amounts to be detected. The relative concentrations of the reacting species mean though that the ozonation reaction pathways of the selected pharmaceuticals will not be affected by the initial concentrations. After ozone treatment of the solutions, the samples were kept in the reactor for at least 20 min to allow for completion of reactions with any dissolved ozone. Control solutions without pharmaceuticals were treated with ozone and analysed in parallel. The data-sets of all experiments were compared to determine the compounds produced by ozonation of the target compounds.

In order to increase the sensitivity of the analysis for the identification of the by-products, samples were pre-concentrated by solid phase extraction (SPE) using cartridges. Sep-Pak cartridges (C18 6 cc vac; 500 mg sorbent per cartridge; 55–105 μm particle size) were purchased from Waters (Rydalmere, NSW, Australia). Prior to pre-concentration, the cartridges were preconditioned with a 7 mL dichloromethane and methanol mixture (1:1 v/v), 7 mL of methanol, followed by 7 mL of Milli-Q water. The samples were loaded onto the cartridges at a flow rate of 1–5 mL/min. The extracted by-products were eluted from the cartridge using 5 mL of methanol at a flow rate of 1–5 mL/min. Thereafter, the eluents were analysed using LC-MS with an injection volume of 20 μL. All samples were performed in duplicate and the maximum error in the concentration of the species is less than 3.0%.

2.4. Analytical methods

The LC-MS used in this study was a Shimadzu single quadruple LC-MS 2020 equipped with electrospray ionisation (ESI) source. ESI is the most applied technique for the determination of transformation products from wastewater in the literature [13–24]. The MS is a universal detector which produces ions that are subsequently isolated according to their mass-to-charge (m/z). To improve the MS outcomes several preliminary experiments were required to optimise the LC-MS parameters.

The target components were separated on a Kinetex® PFP 100A column (100 × 3 mm, 12.6 μm) using a binary gradient made of (A) 0.1% formic acid in Milli-Q water, and (B) acetonitrile at a flow rate of 500 μL/min. The volume of injection was 20 μL. The gradient used was (%B): 0.01 min (10%), 5 min (10%), 20 min (45%), 23 min (90%), 28.10 min (90%), 29 min (10%), 33 min (10%) and 35.01 min controller stop. The column temperature was maintained at 31˚C.

The mass spectrometric data were collected from m/z 100 to m/z 500 in positive and negative ion mode. The cone voltage for each sample was optimised in both positive and negative ion mode (ESI±).

Additional detector parameters were held constant for all samples: interface temperature 350˚C; nebulizing gas flow 1.5 L/min; dry gas flow 3 L/min; DL temperature 250˚C and Heat block 200˚C.

3. Result and discussion

3.1. Effect of ozone dose

Ozone effectively degraded the four selected pharmaceuticals to below the LC-MS detection limit as shown in Fig. 2. The concentrations of all four target compounds sharply decreased with ozone dosage and CBZ, DCF and TMP were completely degraded at an ozone dose of 1.6, 2.3 and 2.8 mg/L, respectively. Each of these three compounds showed rapid degradation at similar rates, suggesting that the formed by-products were more stable to ozone compared to the parent compounds. By contrast, SMX required the largest ozone dose of 4.5 mg/L. As shown in Fig. 2, the SMX concentration rapidly declined from 5 to 3.2 mg/L after consuming only 0.32 mg/L ozone. From this point onwards, the degradation rate steadily declined at a significantly slower rate compared to the initial trend. This slow degradation rate could indicate that the consumption of ozone by other formed highly reactive by-products, instead of SMX. This is supported by the fact that the concentrations of all the SMX by-products are substantially smaller than that for corresponding by-products for the other three pharmaceuticals compounds. The results show that though the four pharmaceuticals react differently, ozonation is nevertheless an effective process for their removal.

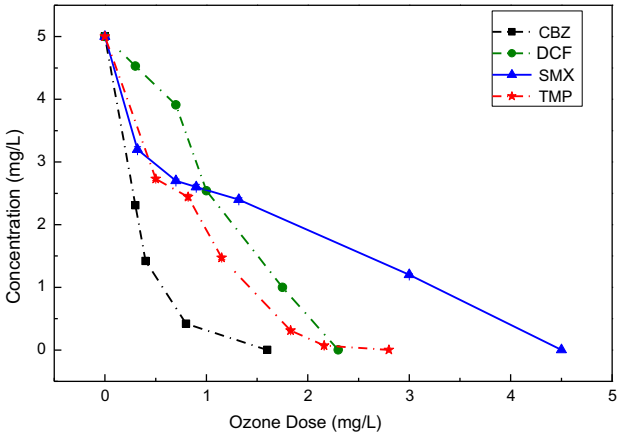

Fig. 2. Removal of CBZ, DCF, SMX and TMP by ozonation as a function of the ozone dose.

3.2. Formation of ozonation by-products

3.2.1. Carbamazepine

Seven ozonation by-products were formed during the reaction of 5 mg/L CBZ with various amounts of ozone (Table 1). As might be expected for an oxidation process the by-products formed had molecular weights higher than the parent compound. This occurred due to the reaction of ozone with the olefin group on the central heterocyclic ring of CBZ, forming an ozonide, followed by cleavage of the double bond [45,46]. The transformation products of the reaction in neutral aqueous solution are usually an alpha-hydroxyalkyl hydroperoxide and a carbonyl compound. Three of the detected compounds (#5, 6 and 7) with molecular masses of 314, 286 and 284 amu, respectively, are reported as CBZ ozonation by-products for the first time in this investigation.

The other by-products (#1, 2, 3 and 4) with molecular masses of 250, 266, 266 and 282 amu, respectively, have been reported as CBZ ozonation by-products previously [29,39]. The present findings confirm the previously proposed structures in the literature, with these by-products having the same mass to charge ratio (m/z) and comparable MS fragmentation patterns as found previously. The mass fragmentation patterns of the most abundant CBZ ozonation by-products were investigated using liquid chromatography electrospray ionisation mass spectrometry (LC-ESI-MS). Collision-activated dissociation of the $[M + H]^+$ molecular ion of compound 1 represented ions at $m/z = 223.2$, 208.1 and 180.1. The mass losses from the $[M + H]^+$ ion were consequently observed to be 28, 43 and 71 amu, respectively. Collision-activated dissociation of the $[M + H]^+$ molecular ion of compound 2 indicated ions at $m/z = 196$ and 180. Mass losses from the $[M + H]^+$ ion were consequently observed to be 71 and 87 amu, respectively. Collision-activated dissociation of the $[M + H]^+$ molecular ion of compound 3 showed ions at $m/z = 249$, 221.1 and 180.1. Mass losses from the $[M + H]^+$ ion were consequently observed to be 18, 46 and 87 amu, respectively. Collision-activated dissociation of the $[M - H]^+$ molecular ion of compound 4 indicated ions at $m/z = 237.2$, 194.2 and 166.2. Mass losses from the $[M - H]^+$ ion were consequently observed to be 44, 87 and 115 amu, respectively. These fragmentation patterns are consistent with the structures found previously. These by-products 1, 2, 3 and 4 have been previously identified as 1-(2-benzaldehyde)-4-hydro-(1H,3H)-quinazoline-2-one (BQM), 1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione(BQD), (BaQM) and 1-(2-benzoic acid)-(1H,3H)-quinazoline-2,4-dione (BaQD), respectively, as shown in Fig. 3 [29,39].

Table 1
The potential by-products produced from ozonation of CBZ as identified by LC-MS

Compound	Retention time (min)	Molecular ion (m/z)		Molecular mass (g/mol)
		$[M + H]^+$	$[M - H]^-$	
CBZ	15.5	237	–	236
1 (BQM)	12.1	251	–	250
2 (BQD)	13.2	267	–	266
3 (BaQM)	11.5	267	–	266
4 (BaQD)	12.9	–	281	282
5	12.0	–	313	314
6	10.9	–	283	284
7	13.3	287	285	286

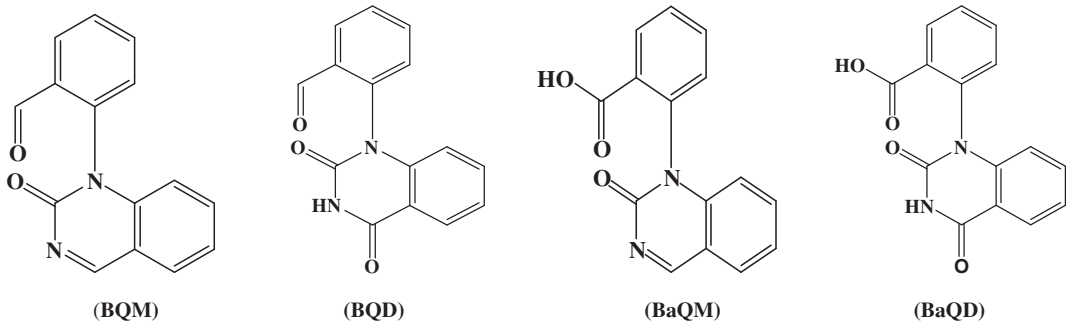

Fig. 3. Major by-products of CBZ [29,39].

The evolution of the major CBZ ozonation by-products at different ozone dosages is shown in Fig. 4. At an ozone dosage of 1.6–1.9 mg/L, approximately 40–50% of the initial CBZ was converted to BQM. The concentration of BQM rapidly increased as the parent compound (CBZ) was degraded by ozone. As CBZ was further removed, the amount of BQM started to gradually decline as a result of the increasing ozone dosage, from 1.9 to 10.4 mg/L. In contrast, the addition of ozone between 10 and 15 mg/L did not cause any further loss of BQM. This suggests that BQM is quite resistant to ozone.

The other four major by-products BQD, BaQD, BaQM and compound #5 were formed at low concentrations compared with the concentration of BQM (Fig. 4). The concentration of the second major by-product (i.e. BQD) was quite high compared to the other CBZ by-products, and could be dependent on BQM degradation. The concentration of BQD increased as BQM gradually degraded. Also, when ozone dosage exceeded 10 mg/L, the concentrations of both BQD and BQM remained stable. Thus, BQM could be an intermediate transformation product of BQD. All these five ozonation by-products were still present even after a dose of 15 mg/L ozone. As these by-products may themselves be biologically active and as toxic as CBZ they may consequently pose an environmental risk.

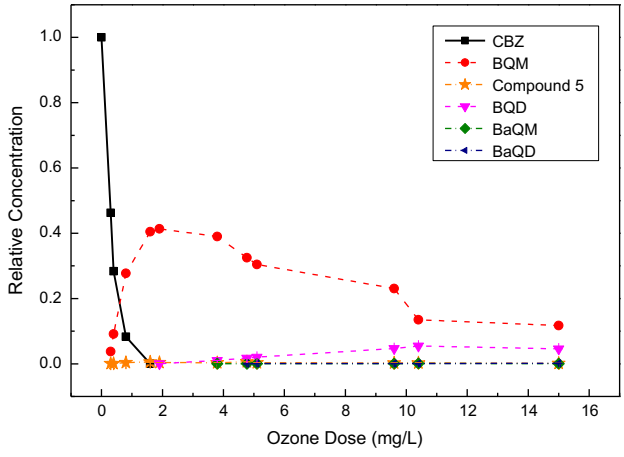

Fig. 4. Evolution of main by-products formed during degradation of 5 mg/L CBZ by ozonation as determined by LC-MS.

3.2.2. Diclofenac

Eleven ozonation by-products were identified during the reaction of 5-mg/L DCF with ozone (Table 2). All of the detected by-products were readily identifiable in the mass spectrum by the existence of the isotopic chlorine atoms in the molecule. Three of the by-products (i.e. #4, 6 and 7) are reported for the first time. This study investigated the fragmentation patterns of the other by-products which have been reported in previous studies [47,48]. The formation of an isomer has been reported in the case of Compounds 1 and 2, named 2-[2,6-dichlorophenyl)-4-hydroxyphenyl) amino]-phenylacetic acid and 2-[2,6-dichlorophenyl) amino]-5-hydroxyphenylacetic acid, respectively. These by-products have the same m/z in the mass spectrum, but different chromatography retention times. The mass fragmentation patterns of the most abundant DCF ozonation by-products were investigated using LC-ESI-MS. Collision-activated dissociation of the $[M - H]^-$ molecular ion of compound 1 represented ions at $m/z = 266.1$, 230.1 and 194.2. Mass losses from the $[M - H]^+$ ion were consequently observed to be 44, 80 and 116 amu, respectively. Collision-activated dissociation of the $[M - H]^-$ molecular ion of compound 2 represented ions at $m/z = 266.1$, 228.1 and 192.1. Mass losses from the $[M - H]^+$ ion were consequently observed to be 44, 82 and 118 amu, respectively. Collision-activated dissociation of the $[M - H]^-$ molecular ion of compound 3 represented ion at $m/z = 160$ which is observed to lose a mass of 140 amu from the $[M - H]^-$ ion. Collision-activated dissociation of the $[M - H]^+$ molecular ion of compound 4 represented ions at $m/z = 282.1$ and 164.1. Mass losses from the $[M - H]^-$ ion were consequently observed to be 44 and 162 amu, respectively. Thus, these findings confirm previously proposed structures in the literature, as these by-products had the same m/z and comparable fragmentation patterns, as shown in Fig. 5.

The reaction of DCF with ozone involved hydroxylation to form a range of by-products. This occur by the formation of compounds whose compositions showed an increase in one or more oxygen atoms with respect to the parent compound, without any changes in the double bond and benzene ring equivalence. For instance, by-products 1, 4 and 5 showed an increased number of oxygen atoms, compared with the DCF. The hydroxylation of the target compound could occur directly via ozone or indirectly via a hydroxyl radical generated from the decay of ozone in the aqueous solution [48,49]. Ozone attacks electrophilic positions on the aromatic ring, and the electron donating groups such as hydroxyl and amine groups induce high electronic density in ortho- and para-positions. Therefore, aromatic rings will react with ozone at these positions. When more than one position in the molecule is susceptible to ·OH attack, the formation of positional isomers can be observed, as in the case of compounds 1 and 2 [50,51].

The evolution of the major DCF ozonation by-products at different ozone doses is shown in Fig. 6. The major by-product of DCF was identified as compound 1 and is consistent with the findings of another study [48]. At an ozone consumption of 1.75 mg/L, approximately 40–50% of the initial DCF was converted to compound 1. As shown in Fig. 6, the amount of compound 1 rapidly increased as the parent compound concentration decreased. When DCF was

Table 2
The potential by-products produced from ozonation of DCF as identified by LC-MS

Compound	Retention time (min)	Molecular ion (m/z)		Molecular weight (g/mol)
		$[M + H]^+$	$[M - H]^-$	
DCF	18.9	–	294.1	296
1	15.6	–	310.1	311
2	15.1	–	310.1	311
3	11.2	–	300.1	301
4	12.8	–	326.1	327
5	10.2	344.1	–	343
6	15.2	310	–	309
7	15.3	342.1	340.1	341
8	18.3	254.1	–	253
9	11.1	–	284.1	285
10	17.5	282.1	280.0	281
11	14.2	298.1	–	297

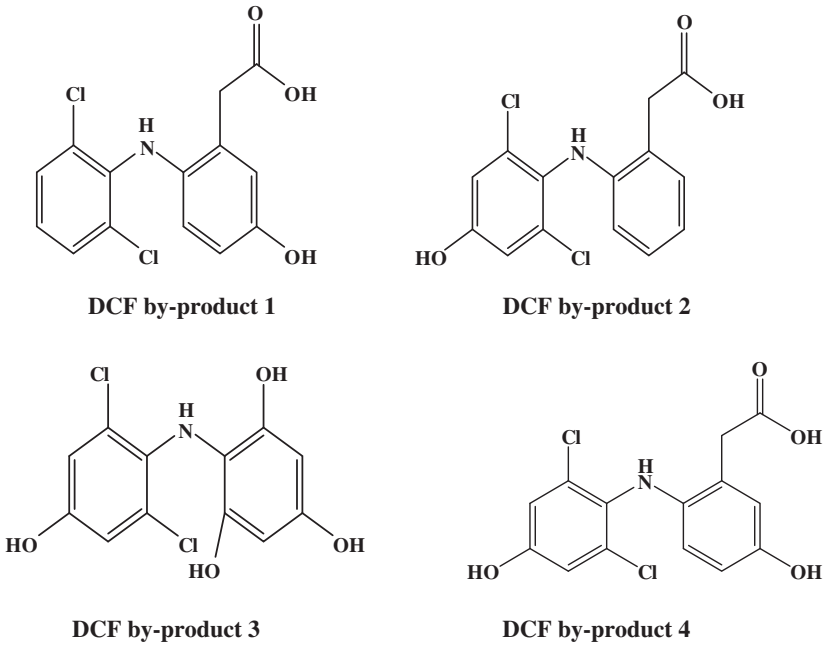

Fig. 5. Major by-products of DCF [47,48].

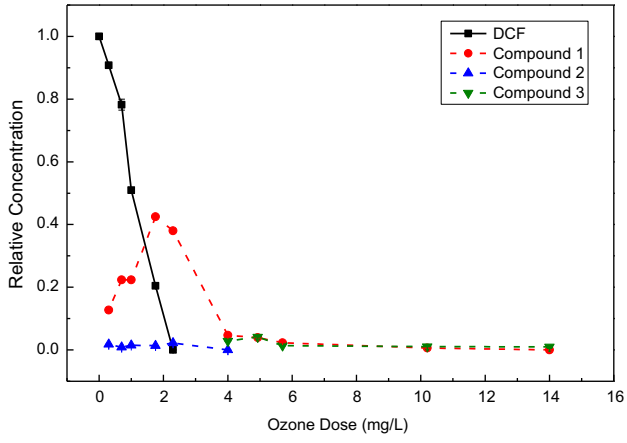

Fig. 6. Evolution of main by-products formed during degradation of 5 mg/L DCF by ozonation as determined by LC-MS.

removed (i.e. at 2.3 mg/L ozone), compound 1 began to gradually decline as a result of the increasing ozone dosing. The concentration of compound 1 was subsequently reduced to low levels; however, it was not fully removed, even at a dose of 14 mg/L of ozone. In contrast, compound 2 which is an isomer of compound 1 was effectively removed as soon as DCF had completely degraded. The third major by-product compound #3 was formed when DCF was removed and compound 1 began to quickly degrade. This compound was still present at a 14 mg/L of ozone, similar to the behaviour of compound #1. Therefore, two of the detected by-products appeared to be more resistant than their parent compound and were not removed even at large ozone dose.

3.2.3. Sulfamethoxazole

Thirteen ozonation by-products were formed and detected during the degradation of SMX by ozonation treatment (Table 3) with six being reported for the first time in this investigation. The SMX ozonation by-products were formed by ozone attack at the amine nitrogen, the aromatic ring, the isoxazole ring as well as N–S bond cleavage [46]. Four of the SMX by-products were detected at a wide range of ozone doses. However, compounds 5–13 were detected at particular ozone doses, and since their concentrations were close to the detection limit, no trends could be identified.

We also confirmed some of the proposed structures in the literature, through comparison of the mass to charge ratio and fragmentation patterns. The mass fragmentation patterns of the most abundant SMX ozonation by-products were investigated using LC-ESI-MS. Collision-activated dissociation of the $[M - H]^-$ molecular ion of compound 2 represented ion at $m/z = 227.1$ which is observed to lose a mass of 42 amu from the $[M - H]^-$ ion. Collision-activated dissociation of the $[M - H]^-$ molecular ion of compound 3 represented ions at $m/z = 186.1$ and 138.2. Mass losses from the $[M - H]^-$ ion were consequently

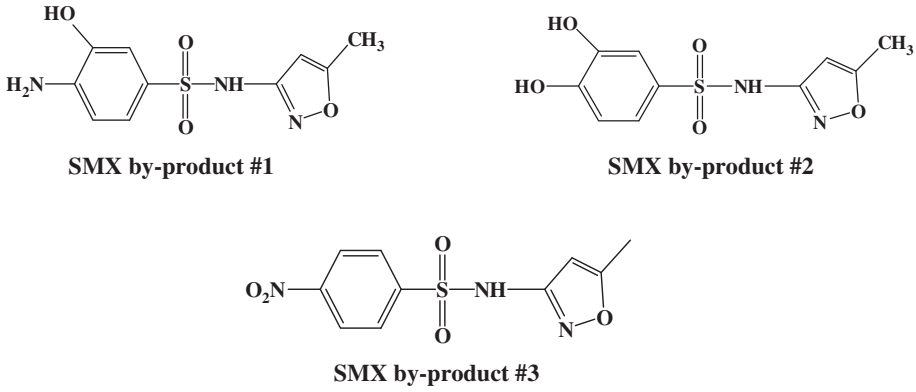

Fig. 7. Structure of major SMX by-products [35].

Table 3
The major by-products produced from ozonation of SMX as identified by LC-MS

Compound	Retention time (min)	Molecular ion (m/z)		Molecular weight (g/mol)
		$[M + H]^+$	$[M - H]^-$	
SMX	10.3	254.1	–	253
1	10.1	270.1	268.1	269
2	10.2	271.1	269.1	270
3	16.9	–	282.1	283
4	6.6	–	282.1	283
5	12.8	–	349.1	350
6	17.3	–	266.1	267
7	11.9	–	280.1	281
8	1.4	–	177.1	178
9	16	–	298.1	299
10	9.7	–	298.1	299
11	11.8	–	226.1	227
12	16.4	–	362.1	363
13	16.8	–	346.1	347

observed to be 96 and 145 amu, respectively. Therefore, the structures of these major by-products were confirmed to be as reported previously and summarised in Fig. 7.

The evolution of the major SMX ozonation by-products at different ozone doses is shown in Fig. 8. All of the detected by-products were present at very low concentrations compared to the initial SMX concentration. At the beginning, any addition of ozone was consumed only with SMX. However, after the formation of by-products the added ozone was likely to be consumed by both SMX and its by-products. This is clearly shown by the slow rate of SMX degradation compared with DCF, CBZ and TMP (Fig. 2). Furthermore, the SMX by-products (Fig. 8) were maintained at very low concentrations compared to the behaviour of CBZ, DCF and TMP by-products (Figs. 4, 6 and 10). Thus, it is likely that SMX by-products are not resistant to ozone and possess not dissimilar reactivity to that of SMX. Only compound 3 remained after 10.1 mg/L of ozone which may imply that it is relatively resistant to ozone, nonetheless the concentration is very low compared to the initial concentration of the parent compound.

3.2.4. Trimethoprim

Fifteen ozonation by-products were identified during ozonation treatment of 5 mg/L TMP (Table 4). Six of them were present at a wide range of ozone dosages, whereas the others appeared at particular ozone dosages and then declined to zero. All of the detected by-products except compound 15 have molecular masses higher than the molecular mass of

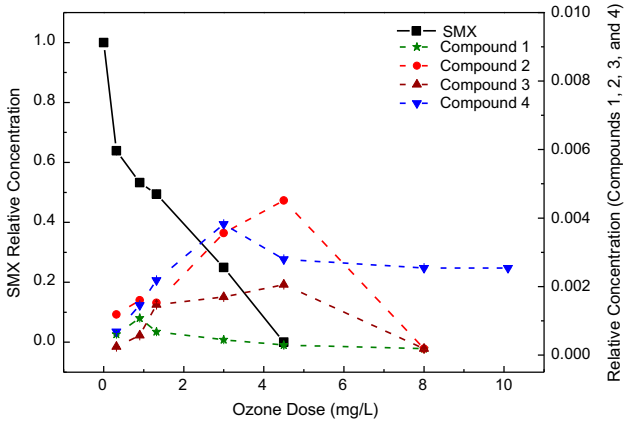

Fig. 8. Evolution of main by-products formed during degradation of 5 mg/L SMX by ozonation as determined by LC-MS.

TMP. Interestingly, six by-products have the same molecular mass of 338 amu, but eluted at different LC-MS retention times through the column. This indicates the formation of a significant number of structural and other isomers, with three of them having similar fragmentation mass patterns. Hydroxylation and carbonylation are the most dominant pathways by which the hydroxyl groups could be connected to either rings of the TMP molecule. Carbonylation may occur in the pyrimidine ring or at the methylene bond [36]. Additionally, a number of oxidation products have been reported previously during the reaction of ozone with TMP [36].

The mass fragmentation patterns of some of the most abundant TMP ozonation by-products were investigated using LC-ESI-MS. Collision-activated dissociation of the $[M + H]^+$ molecular ion of compound #1 represented ions at $m/z = 278.1$ and 181.2. The mass losses from the $[M + H]^+$ ion were consequently observed to be 17 and 114 amu, respectively. Collision-activated dissociation of the $[M + H]^+$ molecular ion of compound 2 indicated ions at $m/z = 181.1$ and 148.1. Mass losses from the $[M + H]^+$ ion were consequently observed to be 144 and 177 amu, respectively. Collision-activated dissociation of the $[M + H]^+$ molecular ion of compound 3 showed ions at $m/z = 307.2$, 275.2, 235.2 and 220.2. Mass losses from the $[M + H]^+$ ion were consequently observed to be 32, 64, 104 and 119 amu, respectively. This is consistent with data and proposed structures in previous reports as shown in Fig. 9.

The evolution of the major TMP ozonation by-products at different ozone doses is shown in Fig. 10. The major by-product of TMP (i.e. compound 1) reached a concentration of 10% of the initial TMP at an ozone dosage of 1.15 mg/L. Subsequently its concentration began to quickly decline as soon as TMP was removed. The second major TMP ozonation by-product (#2) had the same evolution trend, however, it had a lower concentration compared to compound #1. On the other hand, by-products 3, 4, 5 and 6 were formed at almost the same concentration at an ozone consumption from 0.5 to 2.8 mg/L. All of the TMP by-products were completely removed to below their LC-MS detection limit after 4.7 mg/L of ozone was

Table 4
The potential by-products produced from ozonation of TMP as identified by LC-MS

Compound	Retention time (min)	Molecular ion (m/z)		Molecular weight (g/mol)
		$[M + H]^+$	$[M - H]^-$	
TMP	4.5	291.1	–	290
1	13.5	295.2	–	294
2	17.2	325.2	–	324
3	7.7	339.2a	–	338
4	16.9	323.2	–	222
5	9.3	–	339.2	340
6	11.0	–	369.2	370
7	18.9	305.2	–	304
8	4.7	359.2	–	358
9	10.8	355.2	–	354
10	14.7	339.2b	–	338
11	18.7	339.3c	–	338
12	22.0	339.2d	–	338
13	22.7	339.2e	–	338
14	23.4	339.2f	–	338
15	15.4	–	281.2	282

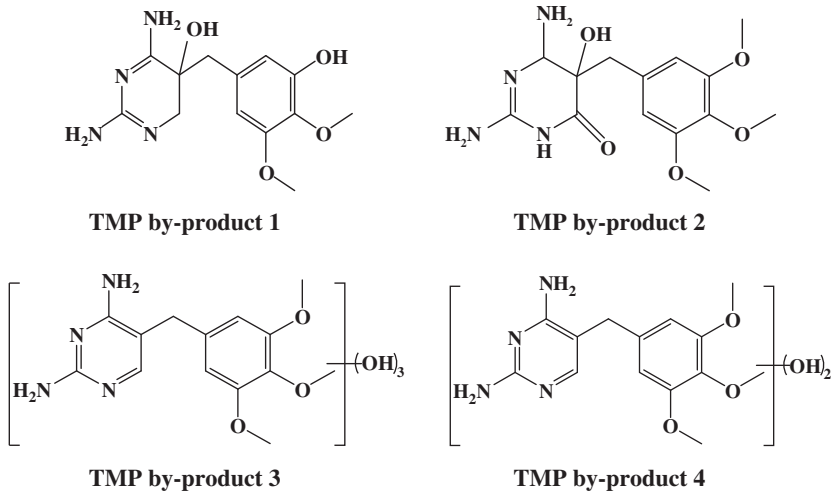

Fig. 9. Major by-products of TMP [36].

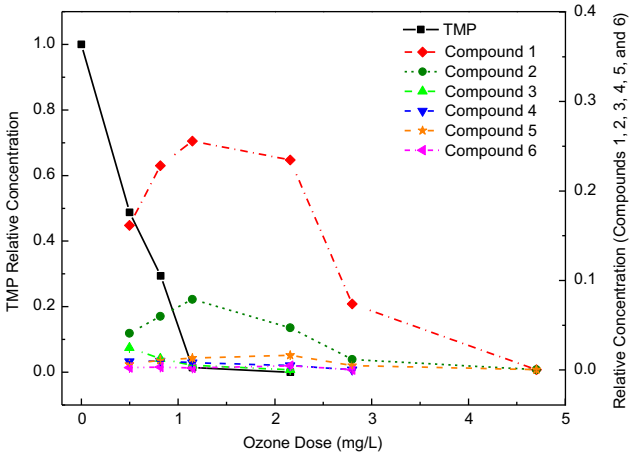

Fig. 10. Evolution of main by-products formed during degradation of 5 mg/L TMP by ozonation as determined by LC-MS.

consumed. This indicates that TMP ozonation by-products are highly susceptible to ozone degradation, similar to their parent compound.

4. Conclusion

This study provides significant new insights into the degradation of CBZ, DCF, SMX and TMP via ozone and the formation of oxidation by-products. Ozonation is an effective process to degrade the selected pharmaceuticals. Various amounts of ozone were required to completely remove 5 mg/L of the target compounds. However, a number of oxidation by-products were identified as forming during the ozonation of all the target compounds. Many of these by-products are reported for the first time. The majority of these by-products are more resistant toward ozone and have higher molecular weights than their parent compounds. For example, a CBZ by-product was persistent at a high concentration, even when the ozone dose was elevated up to 15 mg/L. Thus, measuring only the removal of parent pharmaceutical contaminants from water may not fully represent the treatment efficiency. The detection and identification of emerging by-products produced during the treatment process is essential to accurately assess the removal and fate of pharmaceutical contaminants. Additionally, a combination of other oxidation processes may be an effective way to remove undesired transformation products. Further investigation is being undertaken to carry out detailed structural elucidation of the new by-products as well as to determine whether or not these ozonation by-products are biologically active. Eco-toxicology studies are recommended for new by-products of sufficient interest that are formally identified, extracted and purified. However, this is beyond the scope of the initial phase of the current work.

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