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Analytical approaches to the OH radical induced degradation of sulfonamide antibiotics in dilute aqueous solutions

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ABSTRACT

By combining a large variety of analytical techniques this study aimed at elaborating methods to follow up the degradation of sulfonamides in an advanced oxidation process (AOP): irradiation with ionizing radiation in dilute aqueous solution. In this process, besides other radicals, hydroxyl radicals are produced. As pulse radiolysis experiments show the basic initial reaction is hydroxyl radical addition to the benzene ring, forming cyclohexadienyl radical intermediates. In aerated solutions these radicals transform to peroxy radicals. Among the first formed products aromatic molecules hydroxylated in the benzene rings or in some cases in the heterocyclic rings were observed by LC–MS/MS. Chemical oxygen demand (COD) measurements indicate that at the early reaction period of degradation one hydroxyl radical induces incorporation of 1.5 O atoms into the products. Comparison of the COD and TOC (total organic carbon content) results shows gradual oxidation. Simultaneously with hydroxylation ring opening also takes place. The kinetics of inorganic SO_4^{2-} and NH_4^+ formation, analyzed by ion chromatography, is similar to the kinetics of ring degradation (UV spectroscopy), however, there is a delayed formation of NO_3^-. The latter ions may be produced in oxidative degradation of smaller N containing fragments. The S atoms of the sulfonamides remain in the solution (ICP-MS measurements) after degradation, whereas some part of the N atoms leaves the solution probably in the form of N_2 (total nitrogen content (TN) measurements). Degradation is accompanied by a high pH drop due to formation of SO_4^{2-}, NO_3^- and smaller organic acids. The degradation goes through many simultaneous and consecutive reactions, and with the applied methods the different stages of degradation can be characterized.

1. Introduction

Sulfonamide antibiotics and their metabolites are classified as persistent organic pollutants, due to their resistance to biological decomposition. The degradation in surface waters may take place with the intervention of hydroxyl radicals (•OH). In rivers and lakes •OH is suggested to form in UV photolysis of dissolved organic material or nitrates and nitrites [1]. On sunny days, the •OH formation rates are around 10^{-10} M s^{-1} in the surface layers [2].

Sulfonamides are regularly detected in surface waters, even up to 1 μg dm^{-3} levels, because of their widespread human and veterinary applications [3,4]. A substantial fraction is released to the environment by the wastewater treatment plants. The traditional water purification technologies are not effective enough in the degradation of non-biodegradable compounds [5,6]. Nowadays, a new class of water purification technologies, called advanced oxidation processes (AOP), is under development for degradation of poorly biodegradable organic compounds. Here, also •OH reactions play the key role in degradation [1,7]. Therefore, investigation of •OH reactions, identification of degradation products and study of their toxicity are essentially important for understanding the degradation in the nature and for establishing new water purification technologies. The large number of products that form during degradation and their gradually changing composition during the treatment give a great task to the analytical chemists.

Sulfonamides involved in this study include sulfanilamide (SAA) and seven of its derivatives substituted on the N atom

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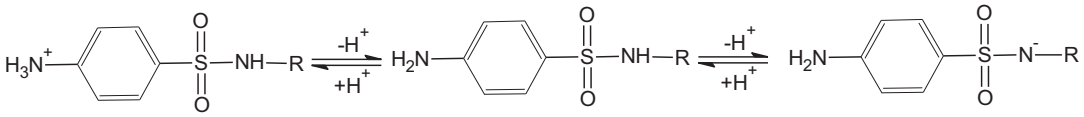
of $-SO_2-NH_2$ by *N*-aminoiminomethyl (sulfaguanidine, SGD), acetyl (sulfacetamide, SCT), 1,3-diazine (sulfadiazine, SDZ), 4,6-dimethylpyrimidin-2-yl sulfamethazine (SMZ), 5-methylisoxazol-3-yl (sulfamethoxazole, SMX), 1,3-thiazol-2-yl (sulfathiazole, STZ) or 3,4-dimethyl-1,2-oxazol-5-yl (sulfisoxazole, SSZ) groups.

Sulfonamides generally have two acid-base dissociation equilibria. At low pH, around 2 the NH_2 group protonates/deprotonates (pK_{a1}), while around pH 5–7, a second protolytic dissociation (pK_{a2}) takes place at the SO_2-NH-R group [8–10]:



$pK_{a1} \approx 2$ $pK_{a2} \approx 5–7$

In our study, ionizing radiation, an AOP technique was applied to produce •OH. During irradiation the radiolysis of water produces three intermediates: •OH (2.8×10^{-7} mol J^{-1}), hydrated electron (e_{aq}^-, 2.7×10^{-7} mol J^{-1}) and hydrogen atom (H•, 0.6×10^{-7} mol J^{-1}) (Reaction (1)) [11,12]. Under practical conditions, when dissolved O_2 is present (aerated solution), e_{aq}^- and H• transform to the O_2^{-}•/HO_2• pair in Reactions (2) and (3) (pK_a (O_2^{-}•/HO_2•) = 4.8). As it is shown in numerous experiments (e.g. [13]), this pair does not contribute directly to the degradation of harmful organic pollutants. In laboratory experiments the reactions of •OH are often investigated in N_2O saturated solution (0.025 M) in order to convert e_{aq}^- to •OH (Reaction (4)). Consequently, in N_2O bubbled solution the active intermediates are •OH and H• with yields 5.5×10^{-7} mol J^{-1} and 0.6×10^{-7} mol J^{-1}, respectively [11].

$$H_2O \rightsquigarrow e_{aq}^- + H^\bullet + {}^\bullet OH \quad (1)$$

$$e_{aq}^- + O_2 \rightarrow O_2^{-\bullet} \quad (k = 1.9 \times 10^{10}\ M^{-1}\ s^{-1}) \quad (2)$$

$$H^\bullet + O_2 \rightarrow HO_2^\bullet \quad (k = 2.1 \times 10^{10}\ M^{-1}\ s^{-1}) \quad (3)$$

$$e_{aq}^- + N_2O + H_2O \rightarrow {}^\bullet OH + OH^- + N_2 \quad (k = 9.1 \times 10^{9}\ M^{-1}\ s^{-1}) \quad (4)$$

Formerly, γ- or pulse radiolysis studies were performed with several sulfonamide compounds [14–22], however, no paper was published on comparing the degradation of a large number of sulfonamides. In a previous publication we reported some of our results on SMX degradation under oxidative and reductive conditions [23].

In this work in a complex approach, the •OH induced degradation products of the mentioned sulfonamides were identified and degradation mechanisms were established. By using pulse radiolysis, the elementary steps of •OH reactions and the short lived, so-called transient intermediates were studied. The stable organic products were separated and identified by LC–MS/MS and UV/vis techniques. For observation of inorganic products ICP-MS and ion chromatographic (IC) techniques were used. During degradation several compounds form as primary products, and the numbers of products increase enormously in the second, third, etc. degradation steps. Therefore, sum parameters, like chemical oxygen demand (COD), total organic carbon (TOC) and total nitrogen (TN) contents were also used to characterize the systems.

2. Materials and methods

All chemicals used in this study were purchased from Sigma–Aldrich. Most of the experiments were conducted with 1×10^{-4} M sulfonamide concentrations.

Pulse radiolysis investigations were carried out using 800 ns pulse width of accelerated electrons and optical detection in 1 cm cell, dose/pulse 20–40 Gy (J kg^{-1}) [24]. In this technique the absorption of energy of a short pulse of accelerated electrons initiates the chemical changes. The formation and decay of short-lived transient intermediates is followed by their light absorption in the UV/vis wavelength range. •OH formed during the pulse reacts with the solute molecules on longer timescale than the pulse time.

γ-irradiations were carried out by a ^{60}Co facility with 6 kGy h^{-1} dose rate. Experiments were carried out at room temperature in unbuffered solutions saturated with air or N_2O.

UV/vis spectra of un-irradiated and irradiated samples were taken by a conventional spectrophotometer (JASCO 550 UV/Vis) with 1 cm cell. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) is one of the primary analytical tools used to investigate pharmaceuticals in environmental samples; it enables both qualitative and quantitative analyses with detection limits below 1 ng dm^{-3}. The method is highly specific with low background interference. The degradation products were separated by an Agilent 1200 liquid chromatograph (LC) equipped with a 2.1 mm × 100 mm Phenomenex Kinetex XB-C18 column. Gradient type elution was used with flow rate of 0.2 cm^3 min^{-1}. Eluents were 0.1% aqueous formic acid (eluent A) and pure acetonitrile (eluent B). Both eluents were filtered through 0.2 μm regenerated cellulose filters. Eluent composition was changed in time as follows: 0 min 3% B, 2 min 3% B, 10 min 50% B, 15 min 3% B, 25 min 3% B. Column temperature was set to 25 °C. The compounds were detected and identified with on-line tandem mass spectrometry (MS/MS). MS experiments were performed both at positive and negative ionization modes using an Agilent 6410 triple quadrupole tandem mass spectrometer with electrospray ionization (ESI). However, positive mode provided significantly better results. In the collision chamber collision induced dissociation with various energies (0–140 V) produces the distinctive product ions. The fragment ions generated were analyzed in the third quadrupole. The ICP-MS measurements were performed on a high resolution, double focusing magnetic sector field inductively coupled plasma mass spectrometer as described previously [23].

NO_3^-, NO_2^-, $SO_3^{2-}$and SO_4^{2-} were identified and quantified with a Metrohm 861 Advanced Compact IC system using a Metrosep A Supp 4-250/4.0 column with 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ buffer solution eluent, while NH_4^+ was determined by a Metrosep C3 column using 5.0 mM HNO_3 buffer solution. Cation and anion detections were carried out at 40 °C and 30 °C, respectively, at 1 cm^3 min^{-1} flow rate.

COD value measures the amount of O_2 needed for total oxidation of organics in the solution. Our technique involves boiling of 30 cm^3 samples at 148 ± 3 °C for 2 h in 8 M H_2SO_4 solution with introduction of $K_2Cr_2O_7$ as oxidizing agent, Ag_2SO_4 as catalyst and $HgSO_4$ for removing chlorides. The non-reacted $Cr_2O_7^{2-}$was removed by titration with Mohr salt using ferroin indicator. The TOC was determined by non-dispersive infrared (NDIR) detection, following a combustion catalytic oxidation method at 680 °C, on a high sensitivity catalyst equipped Shimadzu TOC-L system. The TN measurements were carried out on the same instrument, with a

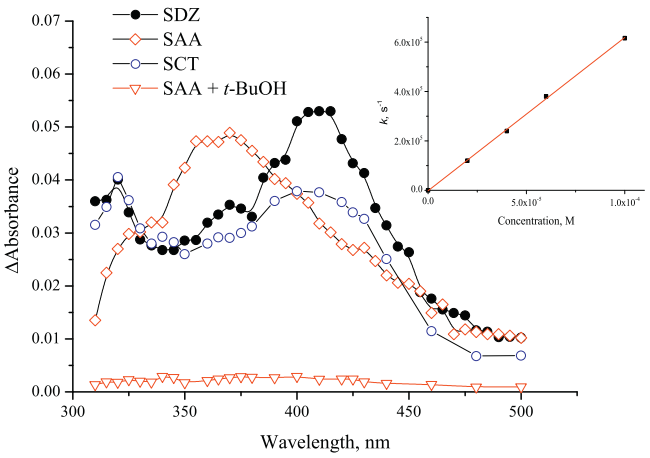


Fig. 1. Pulse radiolysis absorption spectra taken 10 μs after the pulse in N_2O saturated 1×10^{-4} M solutions of SDZ, SAA (without and with *t*-BuOH) and SCT. Inset shows the concentration dependence of the pseudo-first-order rate constant of absorbance build up at 420 nm in SCT solution.

TNM-L measuring unit. The NO_2 (NO was quantitatively oxidized with ozone to NO_2) concentration values were obtained with a chemo luminescence detector, after a catalytic thermal decomposition at 720 °C.

3. Results and discussion

3.1. Pulse radiolysis

For the investigation of transient intermediates, pulse radiolysis experiments were carried out in N_2O saturated solutions. In these solutions the reactive intermediates are •OH and H•, with approximately 9:1 ratio. Fig. 1 shows the absorption spectra of the transients formed in SAA, SCT and SDZ solutions (neutral molecules) (taken 10 μs after the pulse). The maximum (λ_{max}) of the SAA spectrum is at 370 nm, the maxima of the other two compounds are slightly shifted to longer wavelength, to about 410 nm. λ_{max} for SSZ and SGD (not shown in the figure) was at 400 nm. Mezyk et al. [25] published λ_{max} at 400 nm and 415 nm for SMZ and SMX, respectively. The maxima of the molar absorption coefficients in our work and in the literature were found to be in the 2500–4500 M^{-1} cm^{-1} range. In case of sulfanilic acid λ_{max} and ε_{max} are at 385 nm and 4900 M^{-1} cm^{-1} [26], respectively. These absorbances were not observed when •OH was removed by high concentration of •OH scavenger *tert*-butanol (*t*-BuOH, Fig. 1) or isopropanol [14]. The similarities in the absorption spectra and their intensities suggest that the •OH attack mainly occurs at the same place, on the benzene ring, with all molecules.

Since the preferred target of •OH is the benzene ring, hydroxylated aromatic molecules are expected to dominate among the first products.

This view is supported also by the reaction rate constants (k_{OH}) [25]. The k_{OH}'s of the •OH + sulfonamide reactions were measured by the time dependence of the absorbance build-up. These time dependences, the so-called pseudo-first-order rate constants, were determined at several concentrations in the 2×10^{-5}–1×10^{-4} M range. The slope of the pseudo-first-order rate constant-sulfonamide concentration plot supplied the second-order rate constant. This plot is shown for SCT in the inset of Fig. 1. The second-order rate constant is $(5.3 \pm 0.6) \times 10^9$ M^{-1} s^{-1}. Our rate constants, collected in Table 1, reasonably agree with most of the k_{OH}'s determined previously. These k_{OH}'s are very high, that is typical for benzene type molecules. •OH reacts with such aromatics as benzene, aniline or sulfanilic acid, with rate constants of 7.9×10^9 M^{-1} s^{-1}, 8.6×10^9 M^{-1} s^{-1} and 8.2×10^9 M^{-1} s^{-1}, respectively [26,27]. These values are close to the theoretical maximum, the diffusion controlled rate constant, 1.1×10^{10} M^{-1} s^{-1} [27]. The rate constants with the free pyrimidine and the isoxazole rings are smaller: 1.6×10^8 M^{-1} s^{-1} [28] and 3.5×10^9 M^{-1} s^{-1}, respectively [29].

The transient absorption spectrum observed in SGD solution was different from those obtained in the solution of other sulfonamides. In addition to the peak at 400 nm, two more absorption bands appeared in the 300–400 nm range. k_{OH} was the largest for SGD, 9.5×10^9 M^{-1} s^{-1}. We assume that with this compound, beside reaction with the ring, •OH attack on the guanidine part also considerably contributes to the degradation.

3.2. Separation and identification of products

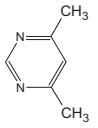
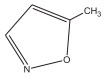
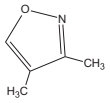
There are several papers in the literature describing different types of LC–MS identifications of products formed in •OH reactions with sulfonamides in advanced oxidation processes [6,8,9,14,18,21,22,30–34]. In our comparative work for product identifications Product Ion mode technique was used with various collision energies to obtain informative fragments and positive ionization mode to achieve the highest ion yield. Basically, samples irradiated at doses between 0.2 and 0.8 kGy were used for LC–MS/MS experiments. All the well measurable products were produced at this dose range and all the products with higher yields were hydroxylated derivatives. At the early stages of the degradation, the products other than hydroxylated ones had 5–10 times lower yields. In addition to the single hydroxylated products, in most of cases double hydroxylated molecules were also observed, albeit with low yields. The most important indicator of the unchanged aromatic ring of sulfonamides is the presence of both 156 and 108 *m*/*z* fragments together (in positive mode ionization) [35]. Their suggested structures are shown in the schemes below (Schemes 1,2 and 3). When these ions are observed in case of singly hydroxylated molecules, the hydroxylation occurs on the R side chain, and not on the ring. This idea can also be applied to the doubly hydroxylated products. In single and double hydroxylated precursor and fragment ions the *m*/*z* values are higher by 16 and 32 mass numbers as compared to the not hydroxylated analogues. This is demonstrated by the example of STZ degradation in Scheme 1. The precursor ion $[STZ+H]^+$ has an *m*/*z* of 256. Upon single and double hydroxylation this value changes to 272 and 288, respectively. The same changes are observed also in the *m*/*z*'s of the two main fragment ions, 156 → 172 → 188, and 108 → 124 → 140. These shifts indicate that hydroxylation occurs on the benzene ring.

Similarly to STZ, in double hydroxylation of SMX also both hydroxyl groups were added to the benzene ring. This was shown by an ion with 99 *m*/*z*, identified as $[NH_2\text{-methoxazole}+H]^+$ that appeared in fragmentation of $[SMX+H]^+$, $[SMX(OH)+H]^+$ and $[SMX(OH)_2+H]^+$, as well.

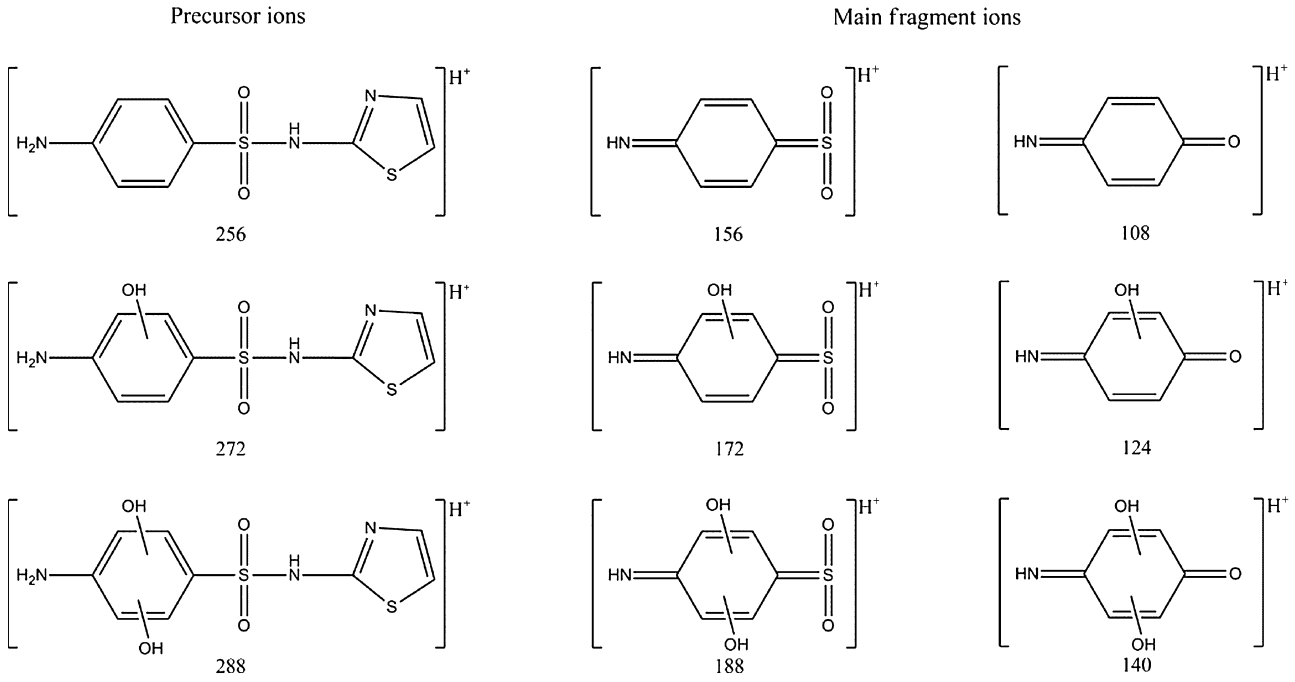
The precursor ions of both SGD and SCT have *m*/*z* of 215, in single and double hydroxylated versions the *m*/*z* increased to 231, and then to 247. Same trends were also observed for the 156 and 108 *m*/*z* ions. It was observed that for SGD the second most intense fragment ion was not the one with 108 *m*/*z*, but an ion with 60 *m*/*z* due to the guanidine fragment, $[(NH_2C(NH_2)NH)+H]^+$. For SAA we detected only single hydroxylation. Probably the hydroxylated product decays very quickly in reactions other than second hydroxylation.

In the case of SSZ the *m*/*z* of the molecular ion, 268, shifts to 284 and then to 300. The 156 *m*/*z* fragment ion transforms to an ion with *m*/*z* of 172 in both single and double hydroxylated molecules. It can be assumed that in the latter case one of the OH's

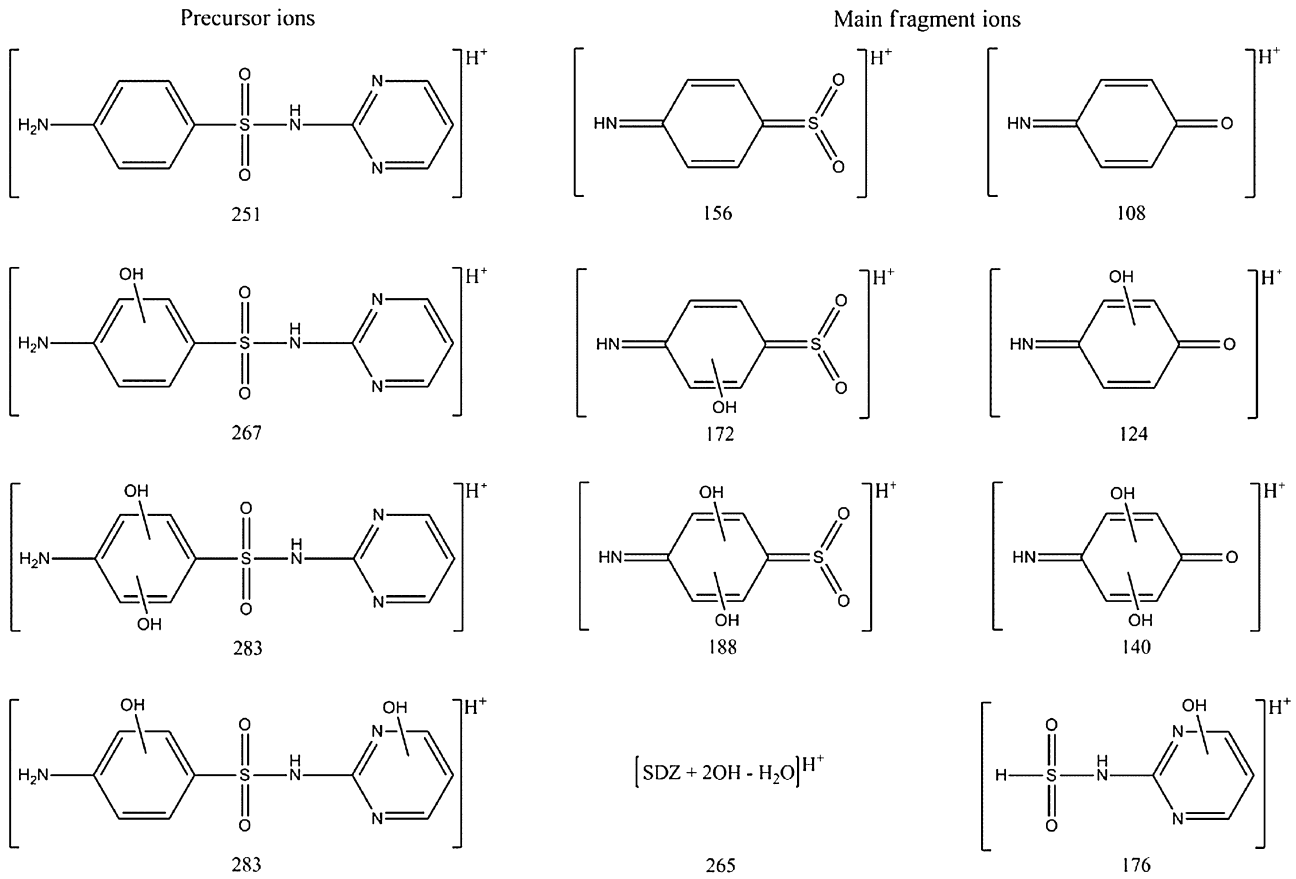
Table 1
Names, abbreviated names used in the text, the R substituent on —SO_2—NHR and characteristic degradation data of sulfonamides investigated. In addition to our rate constants, we collected also rate constants from the literature. The accuracy is generally ±10%.

Sulfonamide abbreviation	Formula	Substituent R	k_{OH}, M^{-1} s^{-1} neutral molecules	COD/dose slope (mg dm^{-3} Gy^{-1})
Sulfanilamide SAA	$C_6H_8N_2O_2S$ MW 172.20 pK_{a1} 2.0, pK_{a2} 10.5	H	4.5×10^9 [a]	4.5×10^{-3}
Sulfaguanidine SGD	$C_7H_{10}N_4O_2S$ MW 214.24 pK_{a1} 1.6, pK_{a2} 11.3		9.5×10^9 [a]	5.6×10^{-3}
Sulfacetamide SCT	$C_8H_{10}N_2O_3S$ MW 214.24 pK_{a1} 1.8, pK_{a2} 5.3		5.3×10^9 [a]	7.6×10^{-3}
Sulfadiazine SDZ	$C_{10}H_{10}N_4O_2S$ MW 250.28 pK_{a1} 2.1, pK_{a2} 6.4		5.7×10^9 [a]	6.4×10^{-3}
Sulfamethazine SMZ	$C_{12}H_{14}N_4O_2S$ MW: 278.33 pK_{a1} 2.6, pK_{a2} 7.4		8.3×10^9 [b]	6.7×10^{-3}
Sulfamethoxazole SMX	$C_{10}H_{11}N_3O_3S$ MW 253.28 pK_{a1} 1.6, pK_{a2} 5.7		8.5×10^9 [b]	5.3×10^{-3}
Sulfathiazole STZ	$C_9H_9N_3O_2S_2$ MW 255.32 pK_{a1} 2.2, pK_{a2} 7.2		7.1×10^9 [c]	7.4×10^{-3}
Sulfisoxazole SSZ	$C_{11}H_{13}N_3O_3S$ MW 267.30 pK_{a1} 1.5, pK_{a2} 5.0		6.6×10^9 [c]	5.6×10^{-3}

[a] Present results.
[b] [26].
[c] [8,9].



Scheme 1. The precursor ions and main fragment ions in the hydroxylation of STZ.



Scheme 2. The precursor and main fragment ions in the hydroxylation of SDZ.

is on the benzene, the other on the oxazole ring. In SDZ solutions the single and one of the double hydroxylations were found on the benzene ring. However, reactions on the benzene and on the pyrimidine rings were also observed (Scheme 2). This was shown by the appearance of the fragment ion 176 *m*/*z*. The main fragment ion had *m*/*z* of 265 [SDZ(OH)$_2$-H$_2$O + H]$^+$).

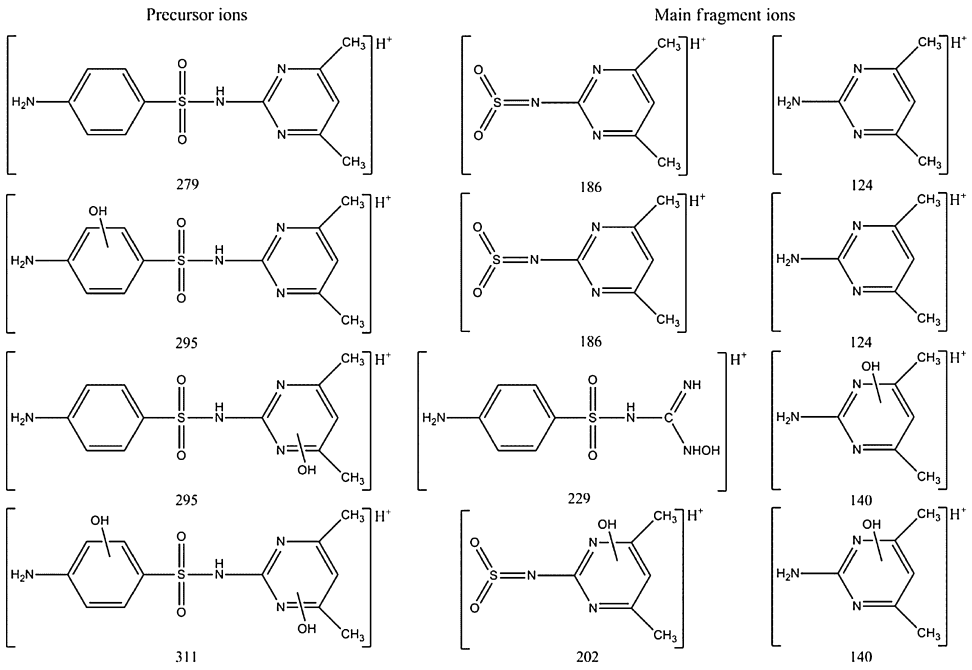
SMZ represents a complicated case as regards both, the degradation pattern of the precursor ion and also the reactions of the SMZ molecule with hydroxyl radical. The precursor ion with 279 *m*/*z* in the collision chamber gives main ions with 186 and 124 *m*/*z*. These fragments contain the heterocyclic ring (Scheme 3). When the benzene ring is hydroxylated, the [SMZ(OH) + H]$^+$ ion gives ions with 186 and 124 *m*/*z* as main fragments. When the heterocyclic ring is hydroxylated the 124 *m*/*z* increases to 140. However, instead of the 186 + 16 ion, an ion with *m*/*z* of 229 was observed as the main fragment ion. Formation of this ion involves degradation of the heterocyclic ring in the MS. Rong et al. [34] observed the same ion in the degradation of SDZ. In the double hydroxylation both rings are substituted with one OH.

The concentrations of hydroxylated products had maxima around 0.6 kGy. By increasing the dose the products disappeared quickly from the solution, above 1.0 kGy they were hardly detectable by the technique used. The product–dose curve was just slightly shifted to higher dose as compared to the decay curves of the starting molecules, which means that the products have similar reactivity with •OH as the starting molecules. The strong decrease in pH (Fig. 2) suggests disintegration of sulfonamide molecules to smaller fragments (mainly acids) [22]. The idea of first hydroxylation and then gradual degradation to smaller fragments is in agreement with the faster decrease of COD than TOC at low doses (see later), and also with literature suggestions [6,31].

3.3. Changes of the sum parameters

Fig. 2 shows the changes in COD, TOC, TN and pH as a function of dose for SAA, SCT and SDZ solutions. The Insets exhibit the dose dependence of the UV absorption spectra. In the dose range between 0 and 1 kGy, the characteristic aromatic UV $\pi \rightarrow \pi^*$ absorption peak with λ_{max} around 260–270 nm gradually disappears. However, in all solutions a new absorption band appears around 300 nm, which must be due to some products (hydroxylated molecules) with altered benzene ring. At low doses, in all the solutions investigated, there is a substantial drop in pH. However, only slight changes of TOC and TN values were observed up to 1 kGy. After prolonged irradiation both values considerably decrease with lower and lower intensity. In contrast, COD decreases also significantly but almost linearly with the dose. Above 2.5 kGy the decrease of COD becomes less intense, as well. Such behavior is consistent with the idea of aromatic ring disintegration under oxidative conditions. In such a case different carboxylic acids form and the pH decreases. In radiolytic degradation of SMZ many different organic acids, like formic acid (maximum concentration at 2 kGy with 2×10^{-5} M), acetic acid (at 2 kGy 5×10^{-5} M) were detected [21]. These acids are known to degrade slowly in •OH reactions (low rate constants, $\sim 10^8$ M^{-1} s^{-1}) [13]. It should also be mentioned, that the formation of inorganic ions strongly influences the pH (see later).

The initial slopes of the COD–dose relations are in the 4.5×10^{-3}–7.6×10^{-3} mg O_2 dm^{-3} Gy^{-1} (1.4×10^{-7}–2.4×10^{-7} mol O_2 dm^{-3} Gy^{-1}) range (Table 1). In aerated solu-



Scheme 3. The precursor and main fragment ions in the hydroxylation of SMZ.

tion with 1 Gy dose 2.8×10^{-7} mol dm^{-3} •OH is introduced into the solution. This analysis also shows that at the beginning of the process the number of O atoms in the products is on average higher by 1.5 than in the starting molecules. The value higher than 1 indicates that even at low doses, in addition to phenols (1 O atom building in), some higher oxidized products also form. The high incorporation is attributed to organic radical reactions with dissolved O_2 and subsequent oxidation reactions [13].

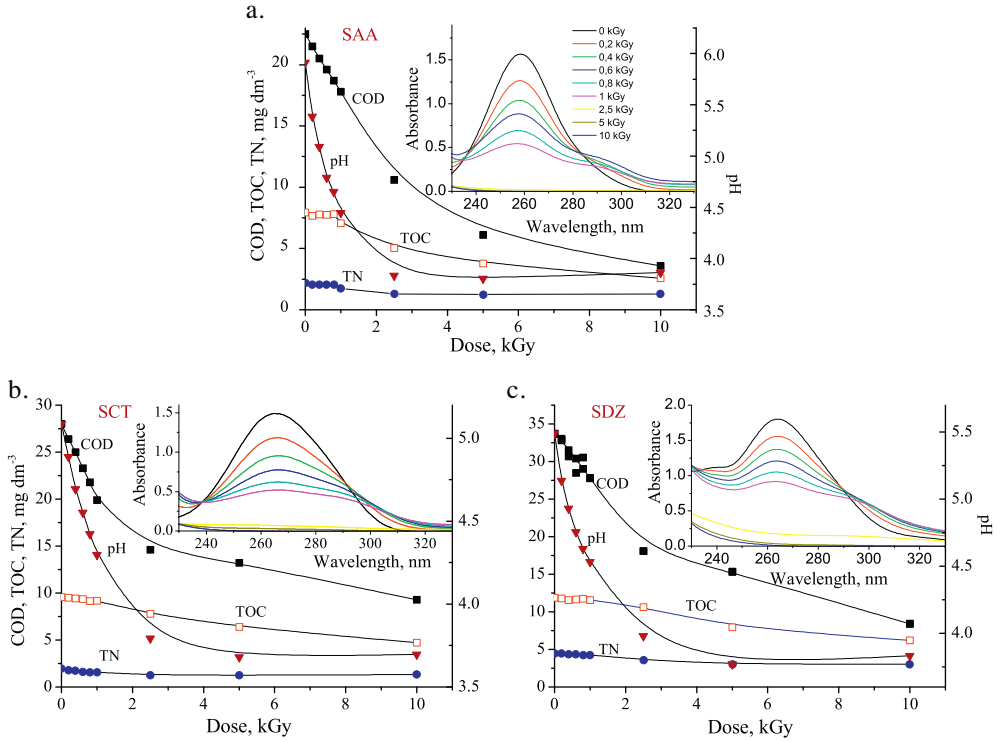


Fig. 2. Dose dependence of COD, TOC, TN and pH in air saturated 1×10^{-4} M SCT (a), SAA (b) and SDZ (c) solutions. Insets: UV absorption spectra at doses between 0 and 10 kGy.

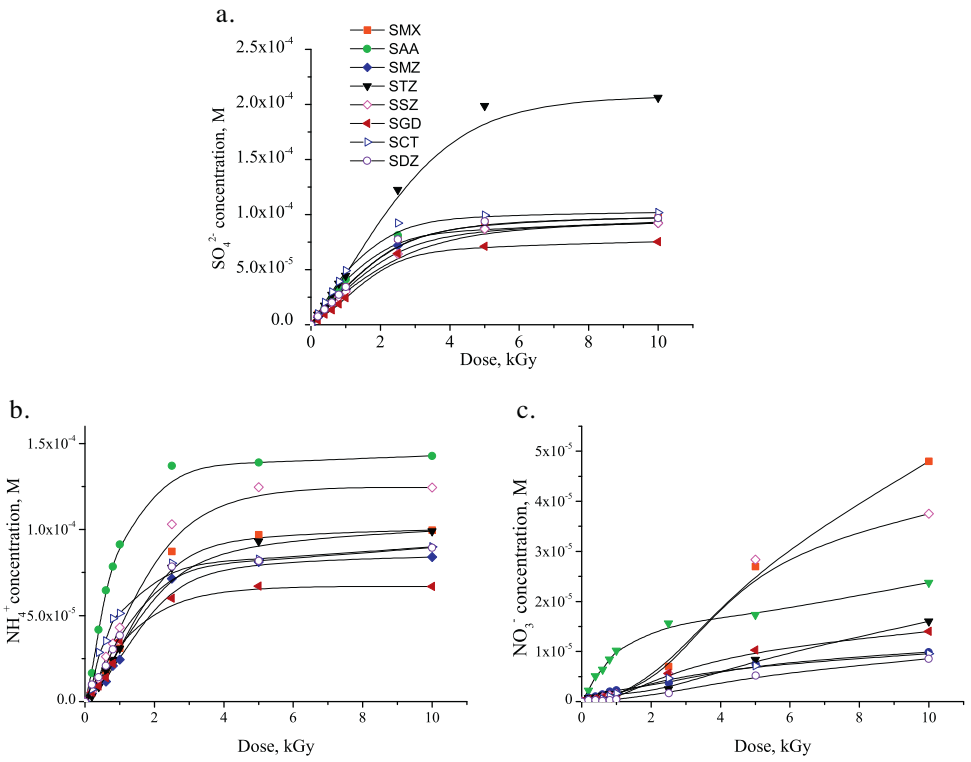


Fig. 3. Dose dependences of SO_4^{2-}, NH_4^+ and NO_3^- concentrations.

3.4. Analysis of inorganic ions

Inorganic ions were analyzed by ion chromatography (SO_4^{2-}, NH_4^+ and NO_3^-), but ICP-MS measurements also helped the analysis of S, while TN measurements contributed to the analysis of N. The quantitative analyses of S and N using ion chromatography and ICP-MS methods allow quantitative comparison of S and N in products and starting molecule. As the ICP-MS measurements show on the example of SMX [23], the sulfur content in the solutions of sulfonamide molecules does not change during irradiation. Similar results are reported in the experiments of Liu and Wang [22] and Liu et al. [21] with SMZ and that of Rong et al. [34] with SDZ. In the ion chromatographic measurements only SO_4^{2-} was detected as S containing ion (Fig. 3a), SO_3^{2-} was not present in measurable quantity. In this experiment the molecules, with the exception of STZ, contain only one S atom. At the highest absorbed doses in most of the solutions about 80–90% of the sulfur is detected in the form of SO_4^{2-} ion. This value is somewhat lower, ~70% for SGD. During radiolysis both S atoms of STZ transform to SO_4^{2-}.

Nitrogen analyses gave more diverse picture. First of all, the TN measurements always resulted in ~20% lower nitrogen content than the calculated value based on the molar concentration and the molecular formula. When these solutions were irradiated, 30–40% of N was not detected in the solution, most possibly it released as N_2. With ion chromatography NH_4^+ and NO_3^- ions were detected (Fig. 3b and c). NO_2^- was not observable here, similarly to the results of Goncalves et al. [36] for SMX.

The NH_4^+ concentration increased with the dose up to c.a. 2.5 kGy, and then leveled off at constant values (Fig. 3b). The rise of the dose dependence curve, however, was slower than the degradation of the starting sulfonamide molecules. This finding indicates that NH_4^+ formation is a complex process and it takes place through several internal steps. From the molecules investigated, SAA and SCT have 2, SMX, STZ and SSZ 3, while SGD, SDZ and SMZ 4 N atoms in their structure. For most of the solutions irradiated with high doses the NH_4^+ concentration is around 1×10^{-4} M, so only 1 N atom transforms to NH_4^+ in 1 molecule. It is obvious to assume that this N atom is in the NH_2 group attached to the ring. In case of SAA solutions the NH_4^+ concentration is around 1.4×10^{-4} M at high doses. The NH_2 group attached to SO_2 in this molecule also has a tendency to transform to NH_4^+ during radiolytic processes.

The kinetics of NO_3^- formation is completely different from that of NH_4^+ production. The NO_3^- concentrations in irradiated solutions are very low below 1 kGy. Although, the concentrations considerably increase above this dose, even at the highest doses the concentrations of NO_3^- are much lower than that of NH_4^+ (except SCT). With most of the molecules only 5–20% of the N content transforms to NO_3^-. NO_3^- formation mainly occurs after the aromatic rings are destructed to smaller fragments.

When a mass balance for nitrogen is made, the NH_4^+ and NO_3^- yields and also the loss of nitrogen, which is estimated from the decrease of TN upon irradiation, have to be taken into account. With these three constituents perfect mass-balance was obtained for SAA. However, for all the other compounds the balance showed 10–30% deficit. It may happen that some N containing products were not detected.

3.5. Dose dependence of mineralization. General evaluation

The analytical techniques applied here allow following up and characterizing the radiation induced degradation of sulfonamide molecules in dilute aqueous solution. This degradation, starting from the intact molecules and going through many intermediate products, finally ends up with inorganic species: H_2O, CO_2, SO_4^{2-}, NH_4^+ and NO_3^-. SO_3^{2-}or NO_2^- are not produced during degradation, probably due to the oxidative conditions. Although, qualitatively many details are understood, much work is still needed on the quantification, qualitative determination of the intermediate products with low yields and on improving the mate-

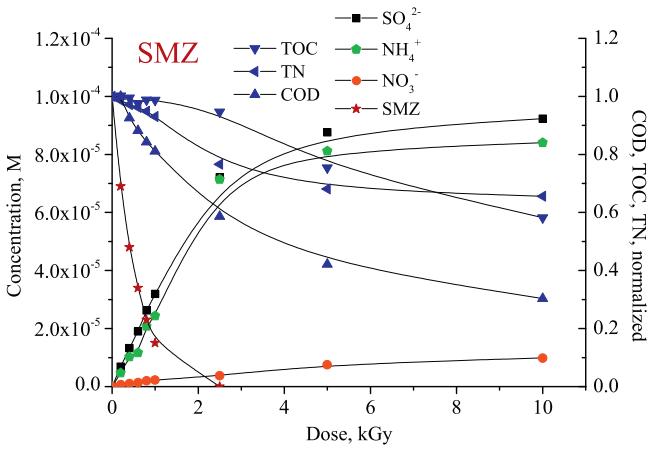
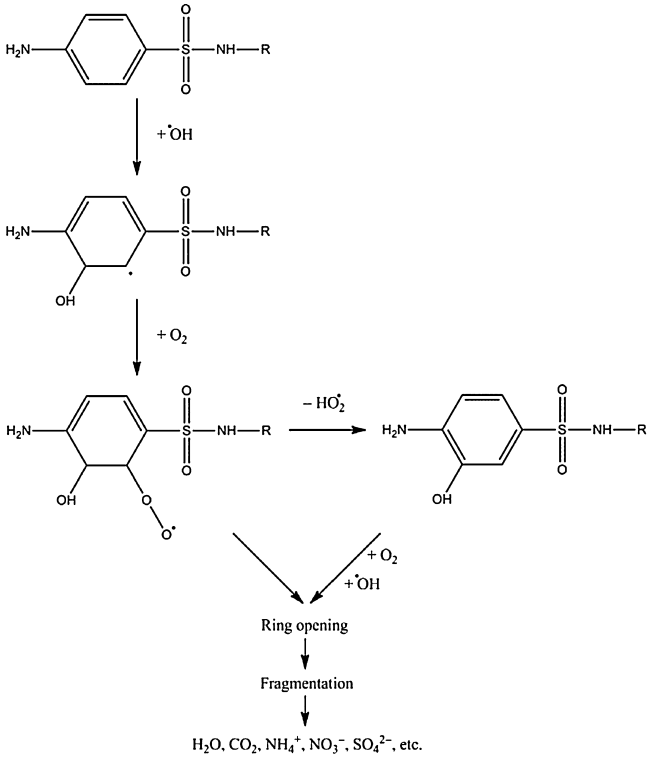


Fig. 4. Comparison of the dose dependences of the degradation of starting molecules, formation of SO_4^{2-}, NH_4^+ and NO_3^- (concentrations) and the normalized COD, TOC and TN values in SMZ degradation.

rial balances. Fig. 4 compares the dose dependences of several measured parameters on the example of SMZ.

As the main process, the degradation starts with •OH addition to the aromatic ring and the cyclohexadienyl radical formed in aerated solutions reacts with dissolved O_2 transforming to peroxy radical (Scheme 4). The rate constant of the cyclohexadienyl + O_2 reaction is rather high, for SCT 1×10^9 M^{-1} s^{-1} is reported [18]. It was demonstrated in numerous publications by von Sonntag et al. (e.g. [37,38]) that peroxy radicals may transform to hydroxylated molecules by HO_2• elimination, or they undergo ring opening to aliphatic compounds. Phenol formation, ring opening and fragmentation occur simultaneously. This is shown by the high value of O atoms building in the products (~1.5) upon one •OH attack and also by the strong decrease of pH and the appearance of fragmented small molecular mass organic acids at low doses. SO_4^{2-} and NH_4^+ products are also observed at low doses, however, their concentration–dose curves are shifted in time as compared to the concentration–dose curves of the intact sulfonamide molecules.



Scheme 4. Reaction mechanism of oxidation.

Phenols disappeared from the solution not much after the decay of the starting sulfonamide molecules. Hydroxylated molecules, due to the aromatic structure probably react with •OH with similar (or because of the electron donating OH group even higher) rate constants as sulfonamides. In LC–MS/MS analyses double hydroxylation was also observed. However, as the UV absorption spectra in insets in Fig. 2 show, up to 1 kGy all the rings undergo oxidative degradation. The SO_4^{2-} and NH_4^+ concentration–dose dependences are similar, these ions may partly form simultaneously with ring decomposition. NO_3^- mainly forms toward the end of decomposition, its source can be the N attached to the SO_2 group, or with molecules having heterocyclic ring, the ring N atoms.

Based on COD, TOC, TN and pH measurements the formations of inorganic molecules/ions (mineralization) are multistep processes that take place simultaneously and consecutively, the molecules first are step-by-step oxidized and finally mineralized.

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