Degradation of pharmaceutical residues in aqueous solutions by ionizing radiation induced hydroxyl radicals

Biologically resistant, toxic organic compounds, like pharmaceuticals pass through the conventional wastewater treatment plants without significant decrease in their concentration. This is especially important in areas, where sewage effluent is released to streams and rivers that are in turn used as a source of raw water for the production of potable supplies for communities living downstream. Concerns have been raised over the potential adverse effects of pharmaceuticals on public health and aquatic environment. Advanced oxidation processes, among them ionizing radiation treatment, combined with conventional, biological methods, are likely to be promising for efficient degradation of pharmaceuticals in wastewater.

The speciality of ionizing radiation treatment is that during water radiolysis reactive intermediates are generated, according to reaction (1), with well-known yields (Spinks and Woods, 1990). In parentheses the yields (*G*-values) are shown in μmol J^{-1} units.

$$H_2O \longrightarrow {}^{\bullet}OH\ (0.28) + e_{aq}^{-}\ (0.27) + H^{\bullet}\ (0.06) \qquad (1)$$

The reactive intermediates can be separated by applying proper conditions (additives). In air-saturated solutions dissolved O_2 reacts with e_{aq}^- (hydrated electron) and $H^{\bullet}$, transforming them to the $O_2^{\bullet-}/HO_2^{\bullet}$ (pK_a= 4.8) pair with 0.33 μmol J^{-1} yield (reactions (2) and (3)). The reactions of $^{\bullet}OH$ and $O_2^{\bullet-}/HO_2^{\bullet}$ may be followed in such circumstances.

$$e_{aq}^{-} + O_2 \rightarrow O_2^{\bullet-} \qquad (2)$$

$$H^{\bullet} + O_2 \rightarrow HO_2^{\bullet} \qquad (3)$$

The e_{aq}^- conversion (4) in N_2O saturated solutions results in doubled yield of $^{\bullet}OH$ (0.55 μmol J^{-1}), while the reactions of both $^{\bullet}OH$ and e_{aq}^- may be followed in N_2 saturated solutions. The e_{aq}^- reactions can be studied in 5% *t*BuOH (*tert*-butanol) containing, N_2 saturated solutions. In such case $^{\bullet}OH$ is scavenged (5), beside e_{aq}^-, *t*BuOH radical ($^{\bullet}$*t*BuOH) of low reactivity is also present.

$$e_{aq}^{-} + N_2O + H_2O \rightarrow {}^{\bullet}OH + OH^{-} + N_2 \qquad (4)$$

$$^{\bullet}OH + (CH_3)_3COH \rightarrow H_2O + {}^{\bullet}CH_2(CH_3)_2COH \qquad (5)$$

The radiolytic decomposition of some pharmaceutical compounds (mainly non-steroidal anti-inflammatory drugs) have already been investigated in our laboratory; results were obtained on the efficiency and pathways of their degradation (Homlok et al., 2011; Takács et al., 2011; Csay et al., 2012; Illés et al., 2012; Szabó et al., 2012). In the present project, the high-energy radiation induced degradation of further pharmaceutical compounds was studied in dilute aqueous solutions. Non-steroidal anti-inflammatory drugs, analgesics and antibiotics (penicillins and sulfonamides) were selected for the investigations as pharmaceuticals most frequently detected in wastewater effluent, surface water and even in drinking water (Lucia et al., 2010, Fent et al., 2006). Based on our results, on a number of compounds of different chemical structure, the degradability was established and a structure – reactivity correlation was suggested for all the compounds studied (Homlok et al., 2013; Homlok, 2015).

Hydroxyl radical induced degradation of maleic acid, fumaric acid and 20 aromatic molecules was investigated in air saturated aqueous solutions. Oxidation was followed up by chemical oxygen demand (COD) and total organic carbon content (TOC) measurements. The dose dependence was found to be linear up to ~30–50% decrease in COD. Most of the ΔCOD/dose-values were in the 7–9 mg dm^{-3} kGy^{-1} range. The oxidation efficiency (*E*) is characterized by the ratio of the number of O_2 molecules built in the products and the number of water radicals ($R^{\bullet}$) introduced into the solution with ρ density (kg dm^{-3}) (6).

$$E = \frac{\Delta\mathrm{KOI/dózis}}{3{,}2\cdot 10^7 \mathrm{G}(R^{\bullet})\rho} \qquad (6)$$

The hydroxyl radical initiated oxidation of phenols, maleic and fumaric acids proceeds with high efficiency; when dissolved oxygen is present the one-electron oxidant $^{\bullet}OH$ induces two-four electron oxidations. When amino, acetamido or hydrazo groups are attached to the ring of phenol the rate is lower, one $^{\bullet}OH$ induces one–two electron

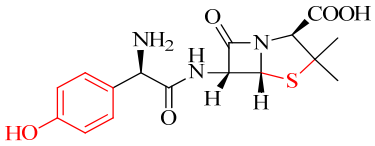
oxidations. The low rate is probably due to intermediate radicals (phenoxy, anilino, semi-iminoquinone, hydrazyl) having low reactivity in their reactions with oxygen.

The change in both toxicity and antimicrobial activity was followed in the course of radiation treatment (Szabó, 2016) in solutions of antibiotics. These compounds are extremely dangerous. Antibiotic resistance is a serious problem worldwide threatening the humankind. The epidemic dissemination of resistance is highly attributed to the selective pressure applied as a result of the widespread use of antibiotics in both human and veterinary medicine (Allen et al., 2010).

There are so called „genetic reactors" in the ecosystem containing diverse bacterial populations under selective pressure that facilitates the exchange of genetic information involving different microorganisms (Baquero et al., 2008). Sewage treatment plants are suggested to be one of them since during the biological process a microbial community and sublethal level of antibiotic concentration are simultaneously present promoting the dissemination of antibiotic resistance. Municipal wastewater treatment plants have been referred to as „hotspots" for spreading antibiotic resistant strains and determinants into the environment (Rizzo et al., 2013). From this standpoint, it is of special interest to eliminate the low antibiotic concentration present in wastewater in order to exclude the spread of wastewater-born antibiotic resistance (Martinez, 2009).

The high-energy ionizing radiation induced oxidative/reductive degradation of penicillin and sulfonamide type antibiotics was followed by various methods. Some of the results will be summarized below.

The structure of penicillins contains both aromatic and thioether moieties susceptible to free radical oxidation as shown on the example of amoxicillin (Scheme 1). Pulse radiolysis technique was applied to study the primary steps of the •OH induced oxidation of amoxicillin (Szabó et al., 2016/1,2). Final products, forming under different circumstances, were identified in order to understand multi-step reactions and to clarify the contribution of each reactive oxygen species to the oxidation process.

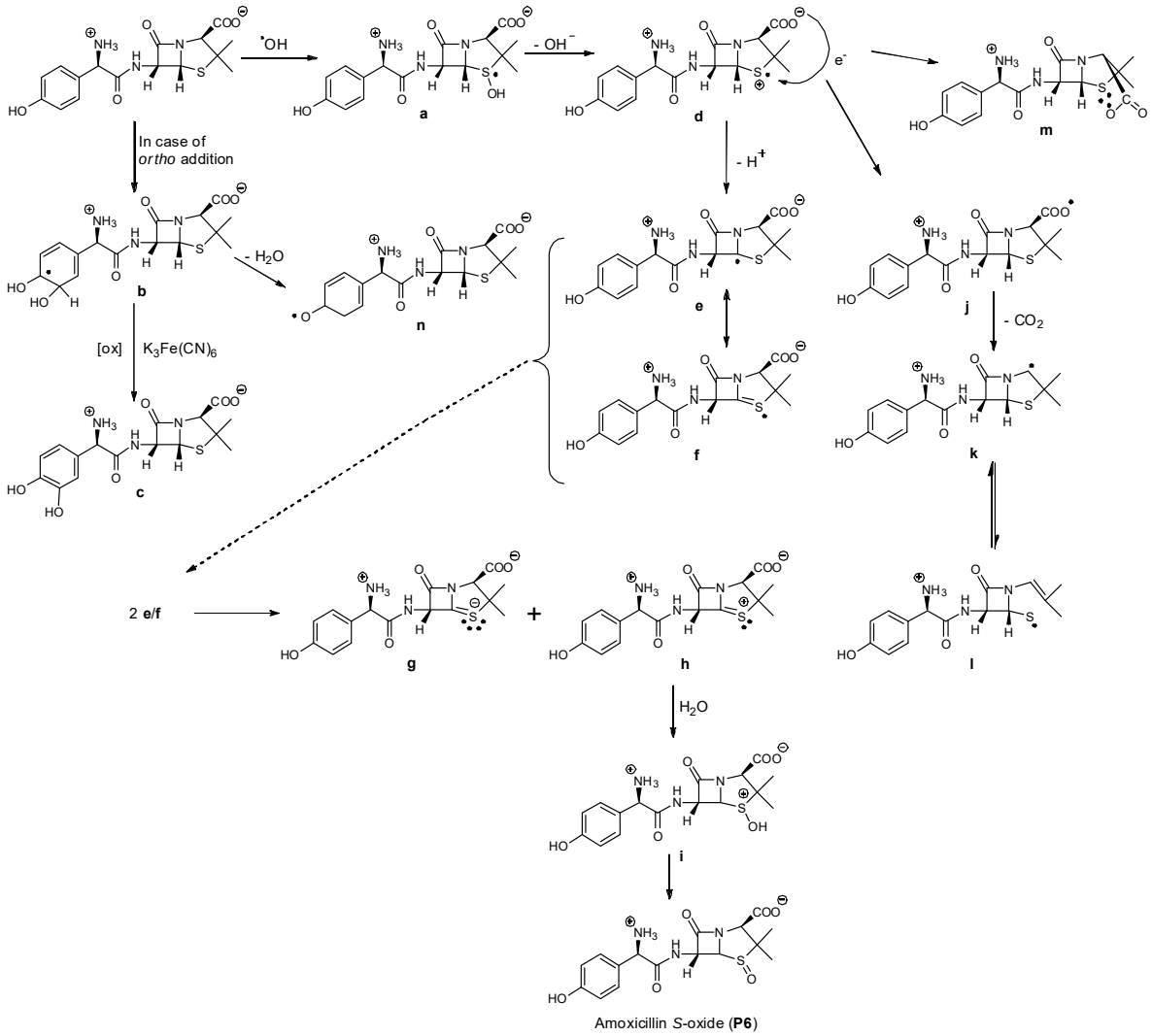


Amoxicillin
(AMX)

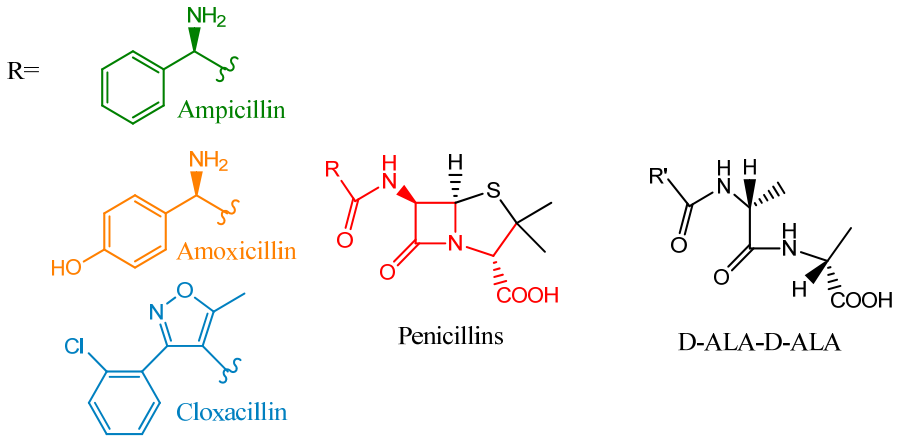
Scheme 1. The structure of a penicillin derivative.

The predominant sites of the •OH attack are suggested to be the thioether group, initially yielding a hydroxysulfuranyl radical adduct, and the aromatic ring (Scheme 2). This hydroxysulfuranyl radical converts to sulfur radical cation, which can transform via three competitive reaction paths: (1) by deprotonation at the adjacent carbon α-(alkylthio)alkyl radicals form, which undergo disproportionation leading presumably to sulfoxide as main product; (2) via the pseudo-Kolbe mechanism it may transform to α-aminoalkyl radicals; (3) the radical cation can be stabilized through intramolecular S.˙.O bond formation. The three-electron bonded dimers of amoxicillin were not formed owing to sterical hindrance. Thiyl radicals were also present in equilibrium with α-aminoalkyl radicals. Aromatic ring hydroxylation occurred along with complex reactions resulting in e.g. oxidation of the methyl groups. The penicillin scaffold is highly affected under oxidative stress. The radical intermediates of sulfur oxidation are reactive species with long lifetime, posing capability for interference with biological processes.

Opening of the β-lactam ring leads to loss of antibacterial activity (Holt and Stewart, 1965). Penicillins target transpeptidases (referred to as penicillin binding proteins), enzymes responsible for cross-links between the peptidoglycan strands in order to confer mechanical strength against the osmotic pressure. Mistake in the specific enzyme-substrate recognition is achieved with penicillins due to the mimicry of the D-alanyl-D-alanine terminus of the peptidoglycan chain (Scheme 3). To follow the destruction of the β-lactam ring a quantitative FTIR method was elaborated based on monitoring the absorbance of the carbonyl stretching vibration on the β-lactam ring at 1766 cm^{-1} using KSCN internal standard with absorption band peaking at 2065 cm^{-1} (Figure 1A). These peaks appeared to be separated from other absorptions.



Scheme 2. Free radical reaction pathway of amoxicillin induced by •OH.



Scheme 3. Mimicry of the D-alanyl-D-alanine (D-ALA-D-ALA) sequence of transpeptidases by penicillins. Model compounds: ampicillin, amoxicillin and cloxacillin.

The removal of the bicyclic system was noted even at low doses in 0.5 mmol dm^{-3} solutions (Figure 1B) for all the three penicillin derivatives (Szabó et al., 2016/4).

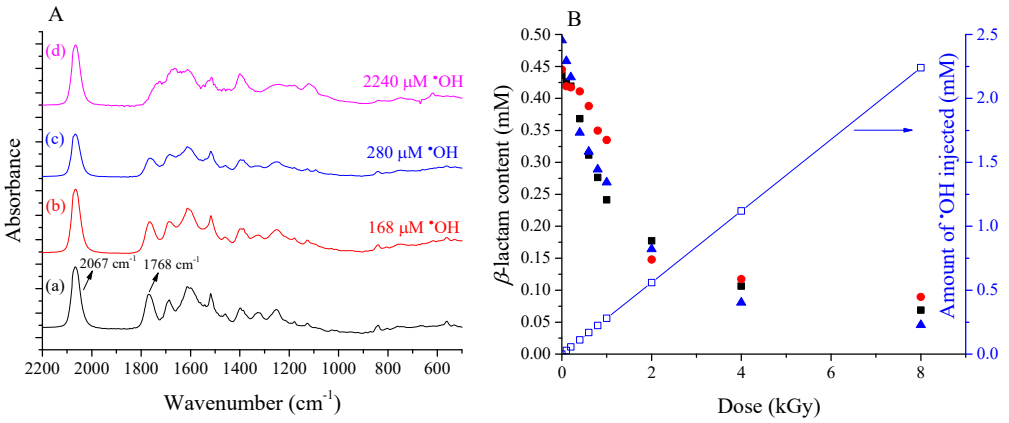


Figure 1. (A) FTIR spectra of amoxicillin, without treatment (a) and after a dose of 0.6 kGy (b), 1 kGy (c) and 8 kGy (d). (B) Quantitative FTIR analysis of the β-lactam content in the case of amoxicillin (■), cloxacillin (●) and ampicillin (▲) as a function of absorbed dose.

The results concerning the change in antimicrobial activity obtained by FTIR method were also supported by microbiological tests. Agar diffusion assay was performed with both Gram-negative (*E. coli*) and Gram-positive bacteria (*S. aureus*, *B. subtilis*) to gain further information on a wider range of strains. *B. subtilis* is revealed to be especially susceptible to the forming products: total loss of the activity was obtained at 8 kGy. Samples with *E. coli* culture exhibited somewhat different dose-dependence. Being less susceptible to the degradation products, the antibacterial potency against *E. coli* has already been lost at ~2 kGy. *Staphylococcus aureus* is an important human pathogen. As a highly capable bacterium of developing resistance to noxious agents, it might be an appropriate candidate for susceptibility testing. 2 kGy was necessary for the removal of its antimicrobial activity.

Growth inhibition test was performed with the marine, Gram-negative luminescent bacterium *Vibrio fischeri* (Menz et al., 2013).

The highest inhibition was observed in cloxacillin solution, above 90% that increased to 100% at low doses (Figure 2). For amoxicillin and ampicillin the inhibition in the untreated solutions was about 20% and about 40%, respectively. However, the inhibition decreased with the dose and low inhibition values were measured for all the three compounds above 2 kGy.

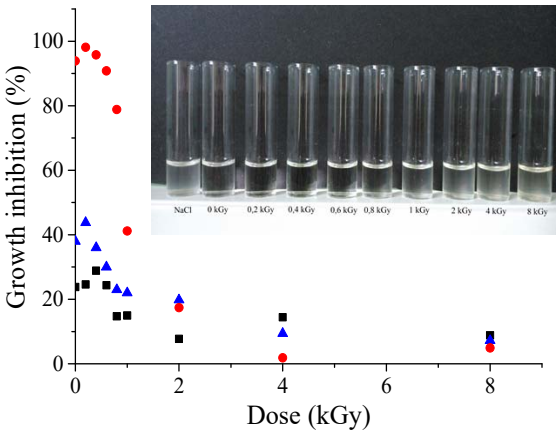


Figure 2. Growth inhibition of *Vibrio fischeri* in amoxicillin (■), cloxacillin (●) and ampicillin (▲) samples as a function of absorbed dose. Insert demonstrates the changes in optical density.

It was shown that e_{aq}^- and $^\bullet OH$ are able to demolish the β-lactam system of penicillins (Szabó et al., 2016/3). The sulfur radical cation, the α-aminoalkyl and α-(alkylthio)alkyl radicals are proposed to play key role in the ring-opening reaction. Attack of e_{aq}^- at the β-lactam carbonyl carbon presumably initiates the opening of the strained four-membered system. Furthermore, it is expected that electron migration occurs from the ketyl radical of the carboxylate moiety towards the β-lactam nitrogen leading to opening of the β-lactam ring. The relatively high efficiency that $^\bullet OH$ provides in elimination of the key β-lactam pharmacophore, which determines the antibacterial activity, is promising in respect to implementation of an advanced oxidation process to eliminate the residual antibacterial activity of wastewater matrices.

Based on these results our main conclusion was that by evaluating the efficiency of advanced oxidation processes in eliminating the antimicrobial activity of a certain drug in relation to wastewater treatment, attention needs to be paid to follow the fate of the pharmacophore that determines the biological activity. Contrary to previous works using kinetics measurements, we found that $^\bullet OH$ and e_{aq}^- are yet good candidates for elimination of the chemical warhead of a penicillin. $^\bullet OH$ attack occurred mainly at the sulfur atom generating reactive intermediates inducing eventually β-lactam ring-opening. Amoxicillin behaved toward e_{aq}^- somewhat like a tripeptide, since electron adducts were observed at the carbonyl carbon and deamination also took place.

The degradation of sulfonamides was studied by combining a large variety of analytical techniques in dilute aqueous solutions (Sági et al., 2015). As pulse radiolysis experiments show the basic initial reaction is hydroxyl radical addition to the benzene ring, forming cyclohexadienyl type 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. 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 results shows gradual oxidation.

For one sulfonamide, sulfamethoxazole (SMX), changes in biological degradability due to ionizing radiation induced decomposition were also studied (Sági et al., 2016). The biological oxygen demand was measured after five days incubation period (BOD_5). The biodegradability of SMX was improved by ionizing radiation treatment with applying a relatively low dose (0.4 kGy). With prolonged irradiation the BOD_5-value further increased, indicating a conversion to biologically treatable substances. The study suggests that already low doses may lead to favourable changes from the point of biological degradability, so the prolonged treatment may become unnecessary and even undesirable. As harmful degradation products may form, the changes in toxic properties should be always considered.

Conclusions

- The biodegradability of pharmaceutical compounds in aqueous solutions can be increased by ionizing radiation treatment with relatively low doses.
- Low degree of mineralization is appropriate to make pharmaceuticals digestible for even low concentrations of microorganisms.
- In solutions containing toxic pollutants with aromatic rings, hydroxyl radical attacks the ring, and the final products are hydroxylated molecules.
- For the solutions of antibiotics studied low doses were appropriate to eliminate the residual antibacterial activity.
- The first degradation products (at low doses) are usually even more toxic than the original compound. Therefore, excessive fluctuations in the treatment efficiency, particularly too low radical exposure should be avoided. This can especially be the case in real wastewater samples where other constituents of the water (natural alkalinity, dissolved organic matter) are expected to scavenge most of the hydroxyl radicals and a relatively low fraction is available for antimicrobial inactivation. Therefore, the advanced oxidation process should be judiciously optimized.

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