Budapest University of Technology and Economics
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Free Radical Chemistry of Penicillin Derivatives

PhD Thesis Summary

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1. Introduction

The term free radical refers to any species containing one or more unpaired electrons. This lone electron, occupying an atomic/molecular orbital, usually confers high reactivity to a species of this type. The omnipresence of free radicals *in vivo* is mainly attributed to the nature of our oxygen-dependent lifestyle. That is, as a result of aerobic metabolism we generate a continuous flow of reactive oxygen species (ROS) and reactive nitrogen species (RNS). A substantial part of free radicals is produced as a kind of accident via the mitochondrial electron transport chain where electron leakage can occur that results in incomplete reduction of oxygen yielding $O_2^{\bullet-}$. These species provide a fountain for the generation of other members of the group: $^{\bullet}OH$ and H_2O_2. $^{\bullet}OH$ is the most powerful oxidant present in biological systems. It attacks biomolecules in close vicinity of its formation unselectively and relatively site-specifically. Nevertheless, $O_2^{\bullet-}$ and H_2O_2 are much less reactive making them able to fulfill specific functions in the cell. Albeit according to the present knowledge they play an essential role in maintaining normal physiological functions *in vivo*, their presence is rather a „two-edged weapon". The pathophysiology of several diseases is linked to the excessive formation of these otherwise conducive agents under circumstances when redox homeostasis is not maintained anymore. This condition is referred to as oxidative stress, under which the antioxidant defense system fails to control the formation of the reactive species. Since this phenomenon is involved in the development of several diseases huge effort is taken to understand the reactions that occur between ROS and biomolecules in the nanoseconds-microseconds timescale.

The oxidative power of $^{\bullet}OH$ inspired many researchers to utilize these agents for trying to solve a devastating issue of the humankind: the environmental pollution. As a result of the social welfare we live in, enormous amounts of chemicals are produced and released into the environment. To maintain a sustainable development the anthropogenic impact must be minimized. To focus on water environment this can be partly achieved by eliminating the pharmaceutical residues that is inevitably released due to the nature of the human metabolism. Without doubt among these agents antibiotic residues are thought to have the most significant impact in terms of mortality affecting most part of the earth. Numerous studies are devoted to aid the implementation of techniques eliminating these residues on the basis of $^{\bullet}OH$ reactions.

Radiation chemistry provides unique tools to study the reactions of free radicals with a compound of interest. This compound may be an environmental pollutant or a biologically relevant chemical entity. The free radical reactions of a special group of antibiotics, namely penicillins, gained appreciable interest in the field of environmental protection and biochemistry. This work aims to answer some of the questions raised in both fields concerning one-electron reduction and oxidation processes.

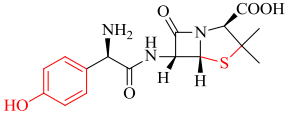
2. Literature review – aims and objectives

2.1. Studying the •OH induced oxidation mechanisms of a model penicillin derivative

•OH, the most powerful agent of ROS, can oxidize almost all kinds of biomolecules at close to the diffusion controlled rate, so practically randomly. However, some selectivity still arises since proteins and peptides are the most abundant constituents of living organisms, and therefore, thought to be initial targets of reactive oxygen species [1]. Among the several targets of oxidants the aromatic ring of tyrosine, the thioether group of methionine and the thiol group of cysteine are the most favored ones and have been implicated in many protective or detrimental processes under oxidative stress [2].

It is apparent from the above considerations that unraveling the mechanisms of the •OH reaction with proteins and following the subsequent fate of the forming radicals are essential for understanding the pathophysiology of several diseases. This is especially important as it provides basis for future drug development. Ongoing studies are aimed at following the early events of the one-electron oxidation of model peptides [3]. However, in proteins the situation appears often to be more difficult to understand owing to their complex nature.

In peptides (like enkephalins) and proteins methionine and tyrosine/phenylalanine residues can be simultaneously present. This further complicates the system since both residues are susceptible to •OH induced oxidation. The co-occurrence of an aromatic and thioether moiety in one molecule can also be found in a certain group of fungal secondary metabolites like penicillins. The peculiar structure of amoxicillin (**Chart 1**), a widely used semisynthetic penicillin derivative, makes it a promising candidate for studying the competing reactions of •OH with these moieties. Furthermore, as pharmaceuticals, penicillins can also be subjected to oxidative stress *in vivo*.



Amoxicillin

Chart 1. Amoxicillin possessing a thioether moiety and a phenolic side chain.

For these purposes the free radical induced oxidation mechanism of amoxicillin was studied by means of radiation chemical techniques. Pulse radiolysis was applied to study the primary steps of the •OH induced oxidation. Final products, forming under different circumstances, were identified by HPLC-MS/MS in order to understand multi-step reactions and to unravel the contribution of each reactive oxygen species to the oxidation process.

[1] Du, J.; Gebicki, J. M. *Int. J. Biochem. Cell B* **2004**, *36*, 2334-2343.
[2] Davies, M. J. In *Encyclopedia of Radicals in Chemistry, Biology and Materials*; Chatgilialoglu, C., Studer, A., Eds; Wiley: New York, 2012; 1425-1458.
[3] Houée-Levin, C.; Bobrowski, K. *J. Proteomics* **2013**, *92*, 51-62.

2.2. One-electron reduction mechanism of penicillin derivatives

Oxidative stress phenomenon connected to bactericidal antibiotics in bacterial and eukaryotic cells is a matter of recent interest [4,5]. It is known that several antibiotics (penicillins as well) interfere with mitochondrial processes and this was proposed to induce ROS generation. The exact mechanism is not specified, however, it might be essential to dispel several debates in this field [6].

In this respect we were particularly interested in the one-electron reduction mechanisms of penicillins. The electrons escaping from the electron transport chain in the mitochondria might induce one-electron reduction of a molecule that can generate reactive species attacking biomolecules or giving the electron to appropriate partners e.g. to O_2. This process might give further information that facilitates the understanding of oxidative stress phenomenon in case of penicillin derivatives.

Therefore, the mechanism of the reaction of e_{aq}^- with model penicillin derivatives, including amoxicillin, ampicillin, cloxacillin, and the 6-aminopenicillanic acid sub-structure (**Chart 2**), was investigated by means of pulse radiolysis techniques.

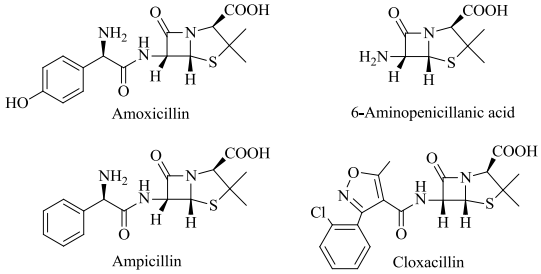


Chart 2. Selected penicillins for studying the one-electron reduction mechanisms.

[4] Dwyer, D. J.; Collins, J. J.; Walker, G. C. *Annu. Rev. Pharmacol. Toxicol.* **2015**, *55*, 313-332.

[5] Kalghatgi, S.; Spina, C. S.; Costello, J. C.; Liesa, M.; Morones-Ramirez, J. R.; Slomovic, S.; Molina, A.; Shirihai, O. S.; Collins, J. J. *Sci. Transl. Med.* **2013**, *5*, 1-11.

[6] Imlay, J. A. *Curr. Opin. Microbiol.* **2015**, *24*, 124-131.

2.3. Elimination of antimicrobial agents from wastewater matrices – towards a sustainable future

Antibiotics in general and penicillins in particular are hazardous water pollutants [7]. Their presence in wastewater facilitates the spread of antibiotic resistance among several bacterial species that has a serious impact on human health. To eliminate the residual antimicrobial activity of wastewater implementation of advanced oxidation processes is recommended [8]. These techniques apply •OH as oxidant.

2.3.1. Demolishing the β-lactam system of a penicillin for eliminating the antimicrobial activity

The β-lactam system is essential for penicillins to exert their antimicrobial activity (it is the so called pharmacophore). Competition is expected to take place between the remote aromatic and thioether moieties for the •OH attack. The outcome of this competition certainly determines the efficiency of the antibiotic inactivation since the thioether group is close to the pharmacophore and the intermediates of one-electron oxidation might eventually induce destruction of this system. Previously, it had been suggested on kinetic grounds that •OH attack occurs mainly at the aromatic side chain of penicillins and the inability of •OH to remove the antimicrobial potency of the molecule has also been concluded [9,10]. However, after evaluating the •OH induced oxidation of amoxicillin (Section 2.1) different picture was emerging for us.

This discrepancy prompted us to investigate the efficiency of elimination of the essential pharmacophore under various circumstances, and to reveal the pathways that might ultimately bring about opening of the β-lactam ring. Therefore, the e_{aq}^- and •OH mediated inactivation of amoxicillin was studied concerning also kinetic aspects with involvement of substructure compounds in the measurements (**Chart 5**).

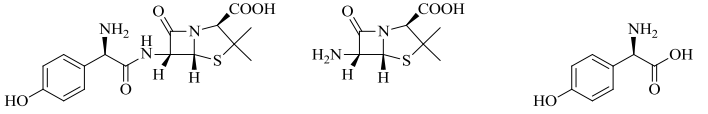


Chart 3. Amoxicillin and its substructure compounds as subjects of this study.

[7] Andersson, D. I.; Hughes, D. *Nat. Rev. Microbiol.* **2014**, *12*, 465-478.

[8] Michael, I.; Rizzo, L.; McArdell, C. S.; Manaia, C. M.; Merlin, C.; Schwartz, T.; Dagot, C.; Fatta-Kassinos, D. *Water Res.* **2013**, *47*, 957-995.

[9] Song, W.; Chen, W.; Cooper, W. J.; Greaves, J.; Miller, G. E. *J. Phys. Chem. A* **2008**, *112*, 7411-7417.

[10] Dail, M. K.; Mezyk, S. P. *J. Phys. Chem. A* **2010**, *114*, 8391-8395.

2.3.2. Effect of the oxidation products on bacterial strains

Advanced oxidation techniques are the methods of choice for elimination of antibiotics from wastewater matrices as long as their high efficiency is taken into account. It should be noted, however, that several studies have shed light on the remaining antimicrobial activity at the beginning of the treatment on account of •OH reactions [11-14].

From this point of view, we studied the effects of the products of the free radical oxidation of model penicillin derivatives (amoxicillin, ampicillin, cloxacillin (**Chart 2**)) on Gram-positive and Gram-negative bacterial strains (**Figure 1**) supplemented by a structure-based chemical approach.

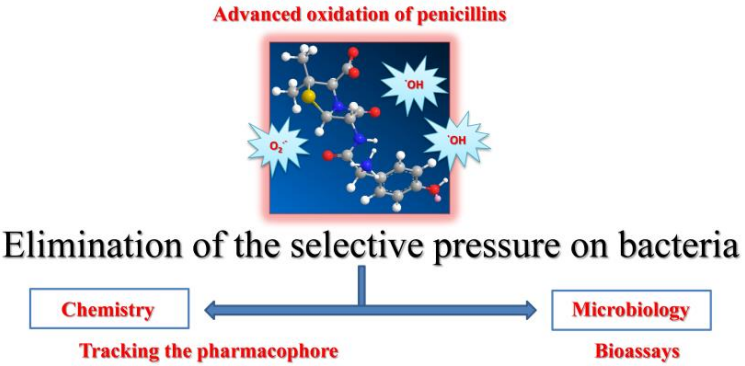


Figure 1. Outlining the experimental approach of the study.

2.3.3. One-electron oxidation mechanism of penicillins in relation to advanced oxidation processes

Unraveling the early steps of advanced oxidation is particularly important to find out the connections between process parameters and the efficiency of the elimination of the antimicrobial potency.

For this purpose, we studied the •OH and $Cl_2^{\bullet-}/Br_2^{\bullet-}$ induced one-electron oxidation of penicillin derivatives (in **Chart 2** and **Chart 3**) using pulse radiolysis techniques. $Cl_2^{\bullet-}/Br_2^{\bullet-}$ forms as a result of the reaction of •OH with Cl^-/Br^-, which are omnipresent in wastewater matrices. $Cl_2^{\bullet-}/Br_2^{\bullet-}$ plays a key role when the treatment of saline or brackish waters are considered [15].

[11] Dodd, M. C.; Kohler, H. P. E.; von Gunten, U. *Environ. Sci. Technol.* **2009**, *43*, 2498-2504.
[12] Dodd, M. C.; Rentsch, D.; Singer, H. P.; Kohler, H. P. E.; von Gunten, U. *Environ. Sci. Technol.* **2010**, *44*, 5940-5948.
[13] Dimitrakopoulou, D.; Rethemiotaki, I.; Frontistis, Z.; Xekoukoulotakis, N. P.; Venieri, D.; Matzavinos, D. *J. Environ. Manage.* **2012**, *98*, 168-174.
[14] Jung, Y. J.; Kim, W. G.; Yoon, Y.; Kang, J. W.; Hong, Y. M.; Kim, H. W. *Sci. Total Environ.* **2012**, *420*, 160-167.
[15] Yang, Y.; Pignatello, J. J.; Ma, J.; Mitch, W. A. *Environ. Sci. Technol.* **2014**, *48*, 2344-2351.

3. Methods

3.1. Irradiation

γ-Radiolysis experiments were performed by exposing the samples of interests to the field of a ^{60}Co γ-source having an activity of 2.3×10^{15} Bq (~ 62000 Ci) (Institute of Isotopes Co. Ltd., Budapest).

Electron pulse radiolysis measurements were conducted using a Tesla Linac LPR-4 type accelerator (TESLA V. T. MIKROEL, Praha, Czech Republic) with kinetic spectrophotometric detection.

3.2. Analytical methods

UV-Vis absorption spectra were taken using a Jasco 550 spectrophotometer.

FTIR spectra were recorded on a Unicam Mattheson Research Series 1 equipment.

^{13}C NMR spectra (500 MHz) were obtained on a Bruker DRX-500 Avance spectrometer.

Products were identified using high-performance **liquid chromatography-tandem mass spectrometry technique** (LC/MS). Transformation products were separated on a Phenomenex Kinetex XB-C18 capillary column (2.1 mm × 100 mm) using an Agilent 1200 liquid chromatograph (LC). The LC system was connected to an Agilent 6410 triple quadrupole MS/MS with electrospray ionization (ESI) interface. MS data were evaluated with Agilent MassHunter Qualitative Analysis software (version 1.3.157.0).

CO_2 loss was calculated from the difference in the **total organic carbon** (TOC) **content** between the irradiated and untreated solutions using a Shimadzu TOC-L equipment.

3.3. Bioassays

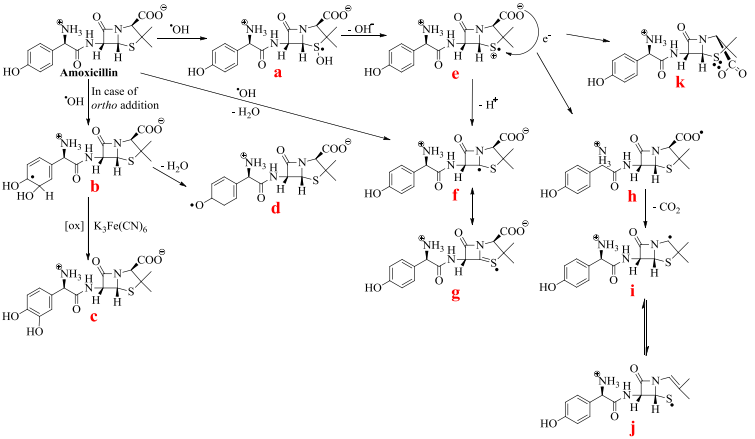
Toxicity tests were performed with *Vibrio fischeri* (Agricultural Research Service Culture Collection, NRRL-B-11177, Hach Lange GmbH, Düsseldorf, Germany) following the DIN EN ISO 11348-2 standard with slight modifications. LUMIStox 300 equipment (Hach Lange GmbH, Düsseldorf, Germany) was applied for measuring the optical density (OD, at 435 nm) and luminescence (acute and chronic toxicity) intensity.

Staphylococcus aureus (American Type Culture Collection (ATCC), ATCC 6538), *Bacillus subtilis* (ATCC 6633), and *Escherichia coli* (ATCC 25922) were used as the reference strains for susceptibility testing. Agar diffusion assay was performed using tryptone glucose agar (TGA) plates. Broth macrodilution assay was carried out using RABIT impedimetric instrument (Don Whitley Scientific, U.K.).

4. Results and discussion

4.1. Free radical induced oxidative transformations of amoxicillin

•OH attack at the thioether group of amoxicillin gives rise to the generation of an OH-adduct at the sulfur atom (**a**) (**Scheme 1**). •OH can also react with the phenolic side chain, in this process dihydroxycyclohexadienyl radical forms (**b**). By using $Fe(CN)_6^{3-}$, this radical can be oxidized (**c**) in a rapid process before the dehydration step leading to the phenoxyl radical (**d**) takes place eliminating this species from the system. The •OH adduct to the sulfur can convert to the sulfur centered radical cation (**e**) via elimination of $^-$OH. α-(Alkylthio)alkyl radicals (**f**) are formed by proton loss from the α-carbon of the sulfur atom of species **e**, these species can be represented with resonance structures (**f** and **g**). The sulfur centered radical cation (**e**) can undergo internal electron transfer with a carboxylate moiety. The electron transfer towards the sulfur atom (leading to **h** in our case) is followed by decarboxylation yielding α-aminoalkyl type radicals (**i**), the process is referred to as pseudo-*Kolbe* reaction. α-Aminoalkyl radicals (**i**) can convert via β-fragmentation to yield thiyl type radicals (**j**) in equilibrium. The sulfur radical cation (**e**) shows high tendency for stabilization via three-electron bond formation by coordination with heteroatoms or with another intact sulfur atom. The three-electron bond ($2\sigma/1\sigma^*$) is created as a result of the overlap between the p orbitals of the unpaired electron of the radical cation and the lone p electron pair of a donor atom (N, O, S). The S.˙.O complex can also be obtained via intramolecular interaction with a sterically available carboxyl oxygen as species **k** indicates. In the light of the calculated radiation chemical yields, it appeared that in amoxicillin the sulfur atom is the predominant site of the •OH attack instead of the aromatic ring.



Scheme 1. Free radical induced transformations of amoxicillin.

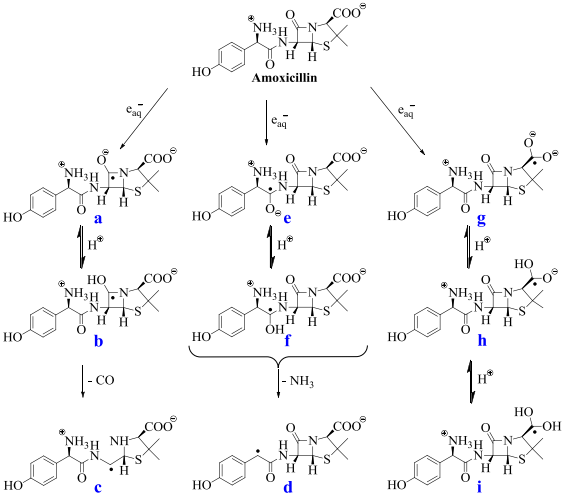
A surprisingly long-living •OH adduct to the sulfur was observed that is unique in the literature. It is suggested that hydrogen bond formation involving the sterically available carboxylate group accounts for this stability.

In final product experiments sulfoxide appeared to be the main product of sulfur oxidation. Dissolved oxygen plays crucial role in the generation of the sulfoxide of amoxicillin, however, in the absence of oxygen H_2O_2 and α-(alkylthio)alkyl radical might govern the reaction pathways *en route* to *S*-oxide.

4.2. One-electron reduction of penicillin derivatives

The one-electron reduction of penicillin derivatives generates ketyl radical intermediates as a result of the accommodation of hydrated electron on the carbonyl carbons One-electron reduction of the β-lactam carbonyl group gives rise to the formation of the corresponding ketyl radical anion (**a**), which immediately transforms to α-hydroxyalkyl radical (**b**) (**Scheme 2**). The unpaired electron is expected to hop to the neighboring carbon forming carbon centered radical (**c**) after CO release and destruction of the β-lactam pharmacophore. Deamination can lead to the corresponding benzyl radical (**d**). We propose that the deamination involves the ketyl radical anion (similarly to peptides) formed as one-electron reduction of the amide carbon (**e**). This species also converts rapidly to the corresponding α-hydroxyalkyl radical (**f**). The initial step in the one-electron reduction of the carboxylate group is the formation of a radical dianion (**g**). This species undergoes step-by-step protonation yielding intermediates **h** and **i**.

The hydrated electron exhibits appreciable reactivity to penicillins, the reaction rate constants were determined to be ~ 5×10^9 mol^{-1} dm^3 s^{-1}.



Scheme 2. One-electron reduction mechanism of amoxicillin.

4.3. Elimination of antimicrobial agents from wastewater matrices – towards a sustainable future

4.3.1. Demolishing the β-lactam system of amoxicillin for eliminating the antimicrobial activity

Contrary to previous studies using kinetics measurements, we have shown that e_{aq}^- and $^{\bullet}OH$ are able to demolish the β-lactam system of penicillins. By using a quantitative FTIR method it was found that $^{\bullet}OH$ and e_{aq}^- eliminates the β-lactam system of amoxicillin with 55% and 84% efficiency, respectively. Since O_2 and $O_2^{\bullet-}$ exhibit enhanced reactivity towards the precursors being involved in ring-opening, a slightly lower efficiency was observed under aerobic conditions. Attack of e_{aq}^- at the β-lactam carbonyl and carboxylate carbon presumably initiates the opening of the strained four-membered system

It was found that the reaction of $^{\bullet}OH$ with the core 6-aminopenicillanic acid nucleus and the 4-hydroxy-D-phenylglicine side chain of amoxicillin occurs with diffusion controlled rate. It was apparent from our competition measurements using KSCN that this technique gives rise to incorrect rate constants in case of an organic sulfide due to the reactivity of the $(SCN)_2^{\bullet-}$ with the sulfur and the formation of S.˙.S dimers absorbing in the same wavelength region.

4.3.2. Effect of the products of penicillins' oxidation on bacterial strains

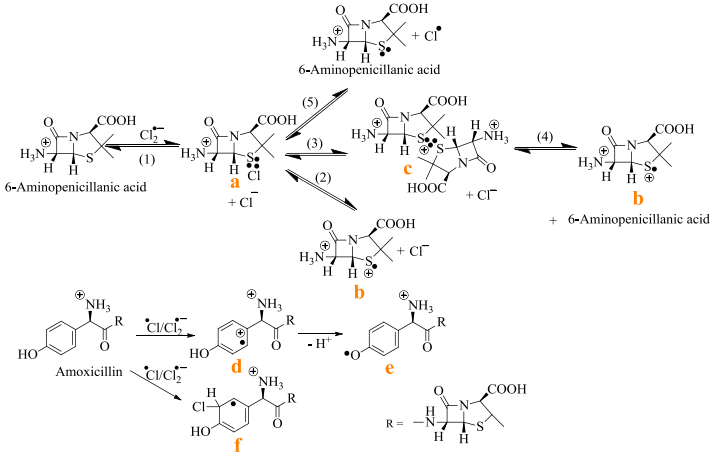
Competition takes place between destruction of the four-membered pharmacophore and reaction at the side-chain aromatic ring. Owing to the multisite attack of $^{\bullet}OH$ several OH-substituted products are generated. These products possess enhanced hydrophilicity that results in a higher diffusion rate through the porin channels of Gram-negative bacteria and through the hydrophilic cell wall of Gram-positive species. Therefore, at low radical exposure the forming products exhibit enhanced toxicity and antimicrobial potency studied with antibiotic susceptibility testing (agar-diffusion, bacterial growth). The adverse effect at high radical exposure presumably arises from the forming polyhydroxylated phenolic compounds. It follows that careful optimization of the advanced oxidation process is necessary.

4.3.3. One-electron oxidation mechanism of penicillins in relation to advanced oxidation processes

The $^{\bullet}OH$ induced oxidation mechanism of other penicillin derivatives, ampicillin and cloxacillin, appeared to be similar to that discussed in case of amoxicillin. The $^{\bullet}OH$ attack occurred again predominantly at the sulfur atom. Whereas in case of 6-aminopenicillanic acid >S.˙.S< three-electron bonded intermolecular complexes were additionally observed, none of the penicillin derivatives followed this reaction pathway on account of the steric hindrance that the side chain aromatic group confers to these molecules.

Penicillins show considerable reactivity towards $Cl_2^{\bullet-}$ with rate constants on the order of 10^9 mol^{-1} dm^3 s^{-1} and similarly high values are predicted for $Br_2^{\bullet-}$. $Cl_2^{\bullet-}$/$Br_2^{\bullet-}$ also targets mainly the thioether moiety.

The reaction of $Cl_2^{\bullet-}$ with the 6-aminopenicillanic acid constituent of penicillins generates a system with a series of equilibria ((1)-(5), **Scheme 3**). In the first step of the $Cl_2^{\bullet-}$ induced oxidation a three-electron bonded adduct is formed (**a**). Due to their instability these species can dissociate forming sulfur radical cations (**b**) (2) or by reacting with an intact molecule yield the >S.˙.S< species (**c**) (3). Species **c** can convert to the sulfur radical cation (**b**) and a 6-APA molecule in equilibrium (4). Since Cl has a higher electronegativity compared to the sulfur the three-electron bonded complex is always thought to dissociate forming the corresponding radical ion (2) instead of undergoing atomic dissociation (5). The sulfur radical cation is a key intermediate, which is a precursor for different radical species (*vide supra*, **Scheme 1**). Therefore, this radical species is also suggested to exist in this system. The reaction of $Cl_2^{\bullet-}$ with phenols involves a one-electron oxidation event yielding the corresponding radical cation (**d**), which converts to the phenoxyl radical (**e**). $Cl_2^{\bullet-}$ exists in equilibrium with $Cl^{\bullet}$. $Cl^{\bullet}$ adds to the aromatic ring forming Cl-cyclohexadienyl radicals (**f**). Since the concentration of $Cl^{\bullet}$ is very low in equilibrium, this reaction is of minor importance. It appeared that ~ 18% of the initially available $Cl_2^{\bullet-}$ reacts with the phenolic side chain of amoxicillin. Contrary to the appreciable reactivity of phenols towards $Cl_2^{\bullet-}$ (~ 10^8 mol^{-1} dm^3 s^{-1} for phenol), the reaction rate constants for benzenes are very low ≤ 1 × 10^6 mol^{-1} dm^3 s^{-1}. Therefore, the effect of the aromatic side chain constituent of ampicillin and cloxacillin when taking the reactions of $Cl_2^{\bullet-}$ into account is assumed to be negligible.



Scheme 3. $Cl_2^{\bullet-}$ induced oxidation of 6-aminopenicillanic acid and amoxicillin at pH 2.

5. Thesis findings

I. We proved that the hydrated electron attack occurs at the carbon atom of the β-lactam and amide carbonyl group, and of the carboxylate group of the penicillin skeleton. The initial intermediates of the one-electron reductions are ketyl radicals. (Paper 1)

II. We found that $^{\bullet}$OH partitioning between the aromatic and thioether moieties in amoxicillin is shifted towards the latter entity. Therefore, the $^{\bullet}$OH induced oxidation of amoxicillin exhibits a reaction pathway typical for oxidation of sulfides. (Paper 2)

III. By unraveling the reaction mechanism of the $^{\bullet}$OH induced oxidation of amoxicillin we found that a short-living and a stabilized long-living $^{\bullet}$OH adduct to the sulfur exist in the system. The long-living species is suggested to be stabilized via a hydrogen bond between the carboxylate group and the OH group attached to the sulfur atom conferring a long lifespan to this intermediate. (Paper 2)

IV. We showed that the hydrated electron and $^{\bullet}$OH are suitable candidates for demolishing the β-lactam system of penicillins. To substantiate our results we conducted IR spectroscopic and kinetics measurements. (Paper 3)

V. We confirmed via parallel final product analysis and microbiological investigations that too low and too high radical exposure during advanced oxidation of penicillins compromises the elimination of the selective pressure on bacterial strains. (Paper 4)

VI. We found by using pulse radiolysis techniques that the one-electron oxidation of penicillins takes place predominantly at the sulfur atom applying both $Cl_2^{\bullet-}/Br_2^{\bullet-}$ and $^{\bullet}$OH as oxidants. Furthermore, we showed that penicillins exhibit enhanced reactivity towards $Cl_2^{\bullet-}/Br_2^{\bullet-}$. A higher sulfur radical cation concentration was obtained by $Cl_2^{\bullet-}/Br_2^{\bullet-}$ compared to $^{\bullet}$OH due to a unique stabilized intermediate that forms as a result of the $^{\bullet}$OH induced oxidation. (Paper 2 and 5)

6. Application possibilities

The unique structure of penicillins made the free radical reactions of these molecules especially peculiar. The one-electron oxidation and reduction of these antibiotics brought many interesting reactions to light adding a new chapter to the chemistry of free radicals and also to the chemistry of antimicrobial agents. The reported reactions can be anticipated to aid the understanding of more complex systems like peptides and proteins with thioether groups in their structure.

Advanced oxidation processes are to be implemented as cutting-edge technologies to treat the wastewater polluted by a wide range of chemicals including antibiotics. In the case of antibiotics a major goal is to eliminate the residual antibacterial activity of the wastewater since it has a detrimental effect on human health through its impact on the microbiota of the environment. Advanced oxidation processes are based on free

radical reactions and rationalization of the ongoing processes under treatment cannot be completed without unraveling the very early events of oxidation. Furthermore, the effect of final products on microbiota at different stages of the treatment is also an important concern of engineering. Both of these issues were addressed in this PhD thesis.

7. Publications

Publications that form the basis of the PhD thesis:

1. Szabó, L.; Tóth, T.; Takács, E.; Wojnárovits, L. One-electron reduction of penicillins in relation to the oxidative stress phenomenon. *International Journal of Molecular Sciences* **2015**, *16*, 29673-29681. [IF: 2.862 (2014)].

2. Szabó, L.; Tóth, T.; Rácz, G.; Takács, E.; Wojnárovits, L. Drugs with susceptible sites for free radical induced oxidative transformations: the case of a penicillin. *Free Radical Research* **2016**, *50*, 26-38. [IF: 2.976 (2014)].

3. Szabó, L.; Tóth, T.; Rácz, G.; Takács, E.; Wojnárovits, L. $^{\bullet}OH$ and e_{aq}^- are yet good candidates for demolishing the β-lactam system of a penicillin eliminating the antibacterial activity. *Radiation Physics and Chemistry* **2016**, *124*, 84-90. [IF: 1.38 (2014)].

4. Szabó, L.; Tóth, T.; Engelhardt, T.; Mohácsi-Farkas, Cs.; Rácz, G.; Takács, E.; Wojnárovits, L. Change in hydrophilicity of penicillins during advanced oxidation by radiolytically generated $^{\bullet}OH$ compromises the elimination of selective pressure on bacterial strains. *Science of the Total Environment* **2016**, *551-552*, 393-403. [IF: 4.099 (2014)]. Independent citations: 1.

5. Szabó, L.; Tóth, T.; Takács, E.; Wojnárovits, L. One-electron oxidation of molecules with aromatic and thioether functions: $Cl_2^{\bullet-}/Br_2^{\bullet-}$ and $^{\bullet}OH$ induced oxidation of penicillins studied by pulse radiolysis. *Journal of Photochemistry and Photobiology A: Chemistry* **2016**, *326*, 50-59. [IF: 2.495 (2014)].

Further articles:

6. Szabó, L.; Tóth, T.; Homlok, R.; Rácz, G.; Takács, E.; Wojnárovits, L. Hydroxyl radical induced degradation of salicylates in aerated aqueous solution. *Radiation Physics and Chemistry* **2014**, *97*, 239-245. [IF: 1.380]. Independent citations: 1.

7. Szabó, L.; Tóth, T.; Homlok, R.; Takács, E.; Wojnárovits, L. Radiolysis of paracetamol in dilute aqueous solution. *Radiation Physics and Chemistry* **2012**, *81*, 1503-1507. [IF: 1.375]. Independent citations: 4.

8. Sági, Gy.; Csay, T.; Szabó, L.; Pátzay, Gy.; Csonka, E.; Takács, E.; Wojnárovits, L. Analytical approaches to the OH radical induced degradation of sulfonamide antibiotics in dilute aqueous solutions. *Journal of Pharmaceutical and Biomedical Analysis* **2015**, *106*, 52-60. [IF: 2.979 (2014)]. Independent citations: 3.

Book chapter:

1. Wojnárovits, L.; Takács, E.; Szabó, L. Gamma-ray and electron beam-based AOPs. In *Advanced Oxidation Processes for Water Treatment: Fundamentals and Applications*; Stefan, M. I., Ed.; IWA Publishing, 2016. In Press.

Conference proceedings (Hungarian):

1. Szabó, L.; Tóth, T.; Rácz, G.; Takács, E.; Wojnárovits, L. Amoxicillin átalakulásai *in vitro* oxidatív környezetben (Transformations of amoxicillin under oxidative conditions *in vitro*). *Őszi Radiokémiai Napok 2014*, 53-57. ISBN 978-963-9970-50-2.

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