IONIZING RADIATION INDUCED DECOMPOSITION OF ANTIBIOTICS IN WASTEWATER

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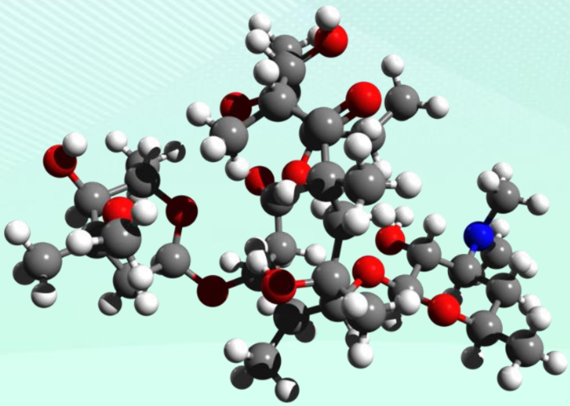

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Pharmaceuticals in the aquatic environment

Wide varieties of toxic organic compounds are entering the aquatic environment. The main sources of these impurities are the wastewater treatment plants for domestic sewage. The co-occurrence of sublethal antibiotic concentration and high dense of microbial population provides ideal condition for facilitating the selection and propagation of resistant bacteria in sewage treatment plants. Conventional methods in sewage treatment plants are not able to completely eliminate the antibiotics. The effluent emitted to the surface waters contains pharmaceuticals and bacteria.

Biodegradation of antibiotics. Results of closed bottle test.[a)]

Test compound	Concentration µg ml^{-1}	Biodegradation after 28 days, %	Biodegradation after 40 days, %
Cefotiam dihydrochloride	4.8	7	10
Ciprofloxacin	3.5	0	0
Metropenem	2.5	7	7
Penicillin G	3.0	27	36
Sulfamethoxazole	3.8	0	0

a) Al-Ahmad, A., Dashner, F.D., Kummerer, K., Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G and sulfamethoxazole and inhibition of waste bacteria. Archiv. Environ. Contam. Toxicol. 37, 158 (1999)

Pharmaceuticals in the aquatic environment

How to solve this problem?

Post treatment of the wastewater treatment plant effluent by ionizing radiation.

↓

Elimination of the antimicrobial activity and deactivation of the bacteria in one step.

Radiolysis in aqueous solutions

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$H_2O \longrightarrow {}^{\bullet}H, e_{aq}^{-}, {}^{\bullet}OH, H^{+}, OH^{-}, H_2O_2, H_2$

$G({}^{\bullet}H) = 0.06\ \mu m\ J^{-1}$; $G({}^{\bullet}OH) = 0.28\ \mu m\ J^{-1}$; $G(e_{aq}^{-}) = 0.28\ \mu m\ J^{-1}$

The purpose of our work is:

- To describe degradation mechanisms and determine rate constants for the reactions of the water radiolysis intermediates with the pollutant molecules.
- To suggest the dose necessary to eliminate the toxicity and biological (e.g. antimicrobial) activity of pharmaceuticals.

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Mechanism of free-radical induced oxidation

- Pulse radiolysis
  - Tesla Linac LPR-4 accelerator (4 MeV)
  - Pulse width 800 ns
  - Dose/pulse 20-40 Gy
- Final product analysis
  - ^{60}Co facility with 11.5 kGy h^{-1} dose rate
    - LC/ESI-MS analysis
      - Agilent 1200 liquid chromatograph
      - Agilent 6410 triple quadrupole MS/MS with electrospray ionization (ESI) interface
    - CO_2-release using a Shimadzu TOC-L equipment

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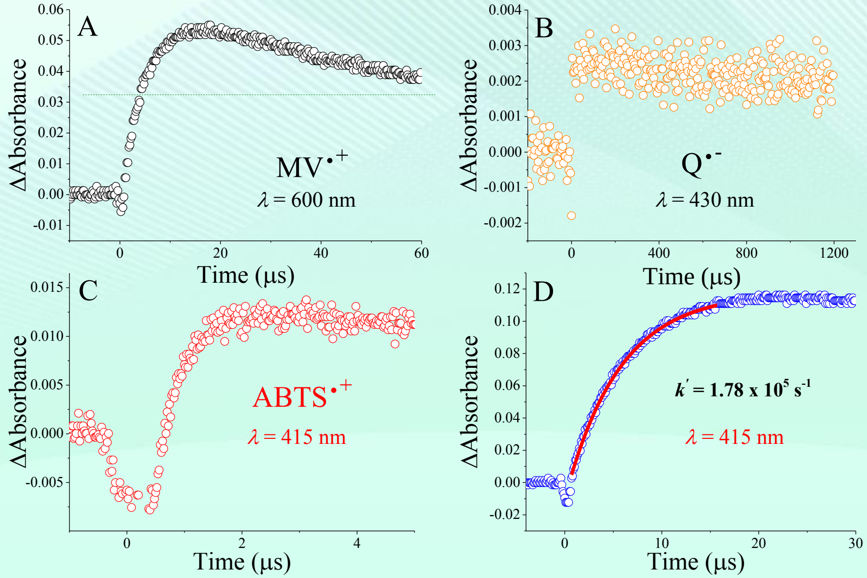
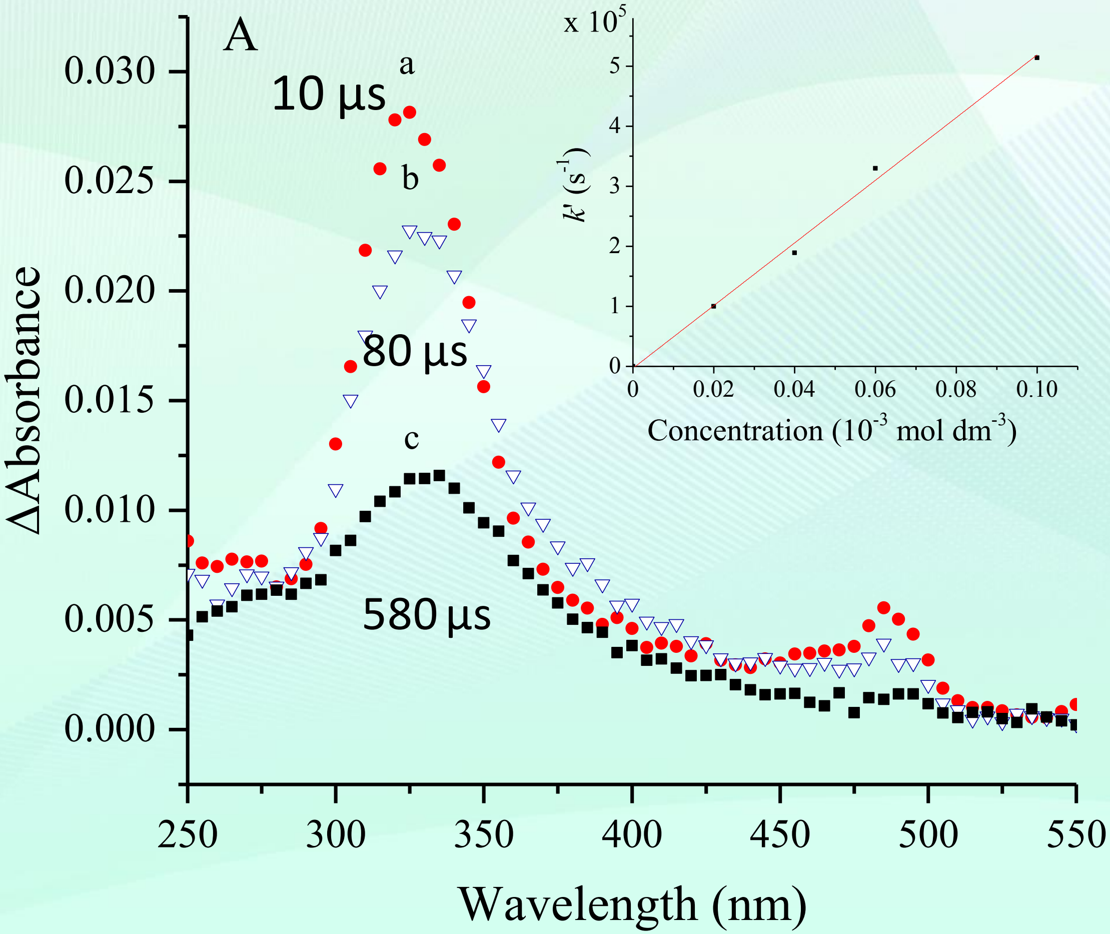
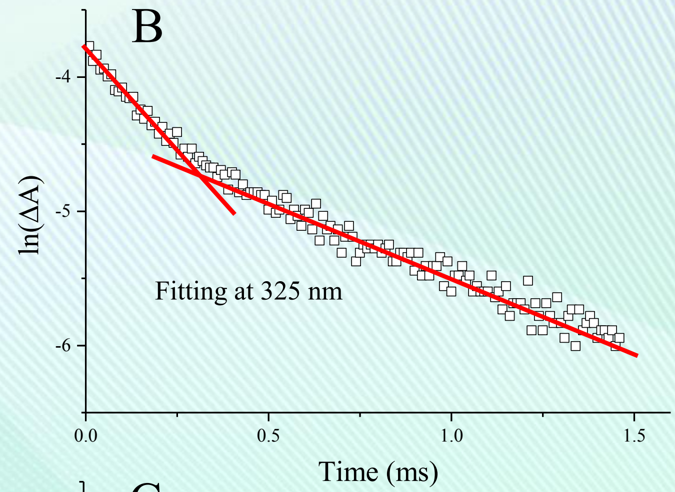
Decomposition kinetics and mechanism of free-radical induced degradation

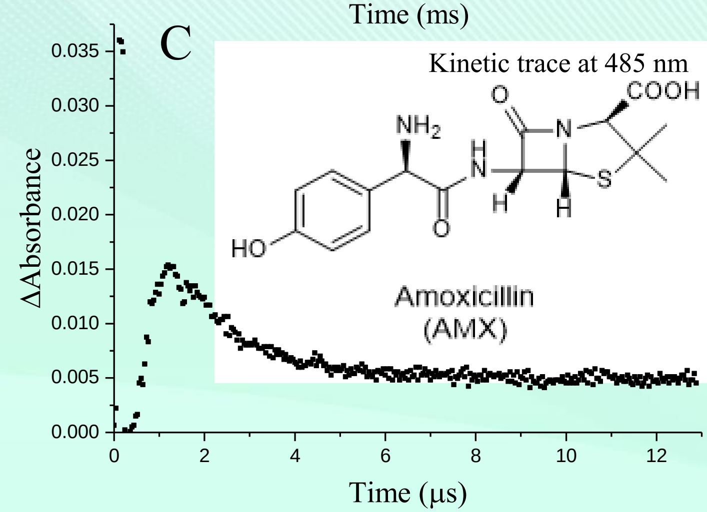


Decomposition kinetics of penicillin derivative (β-lactam) antibiotics

Reaction of e_{aq}^-







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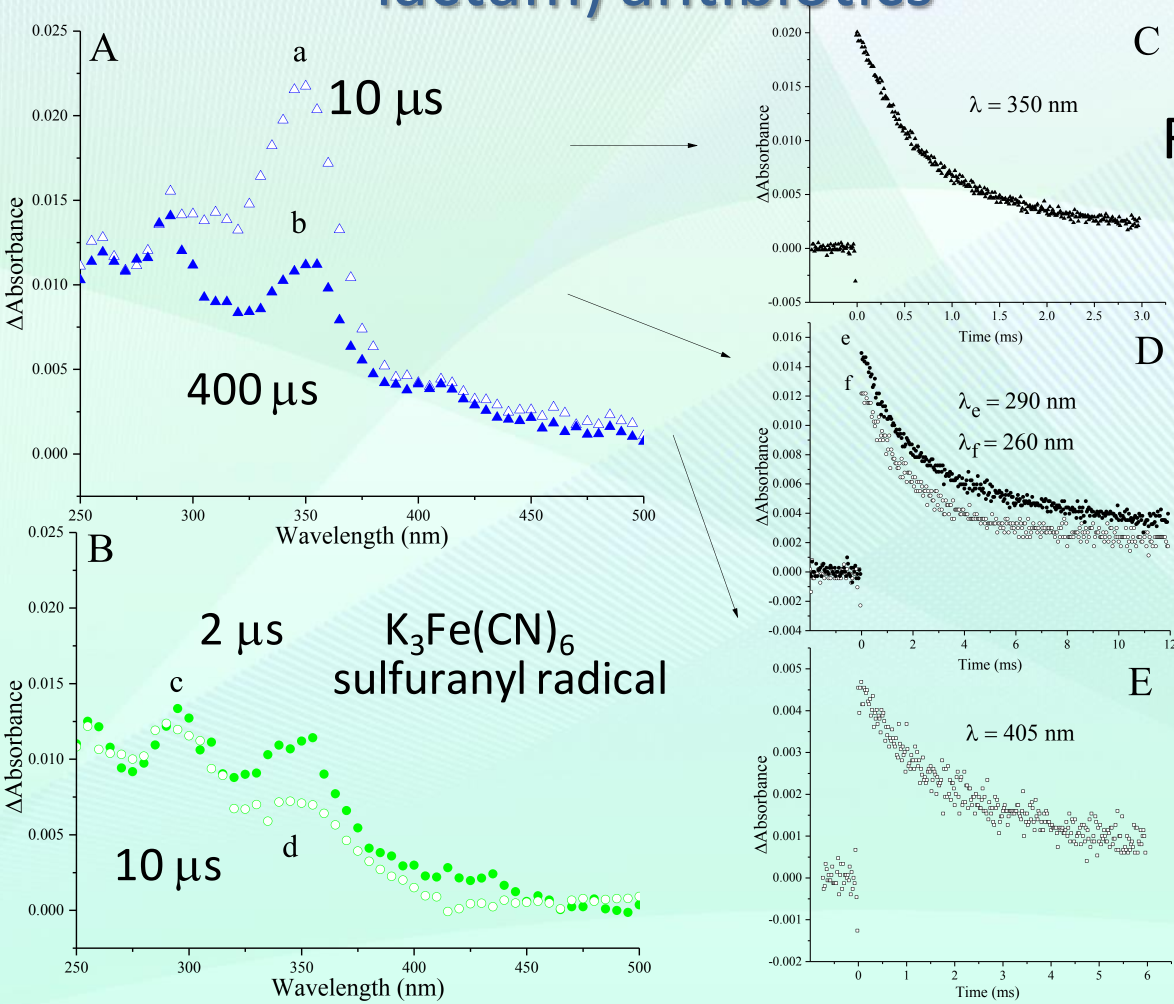
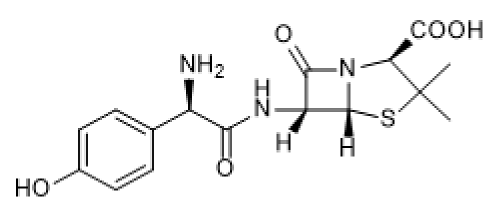
Transient absorption spectra of intermediates in e_{aq}^- reaction in N_2 saturated AMX solution

Decomposition kinetics of penicillin derivative (β-lactam) antibiotics



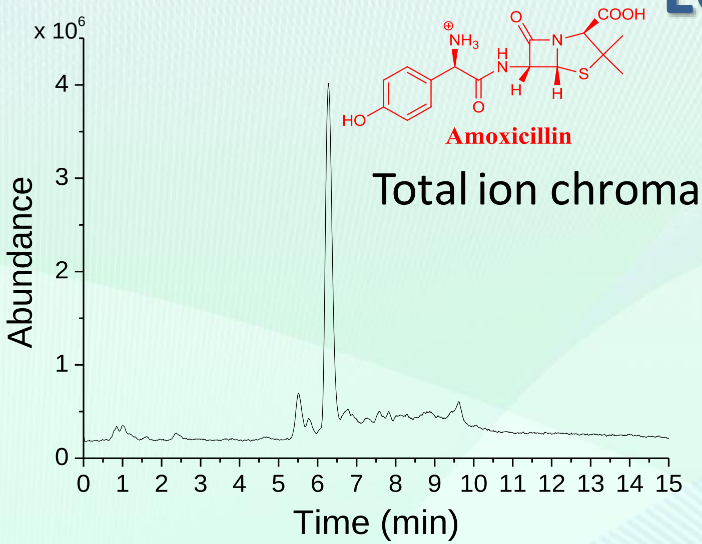
Reaction of •OH radical



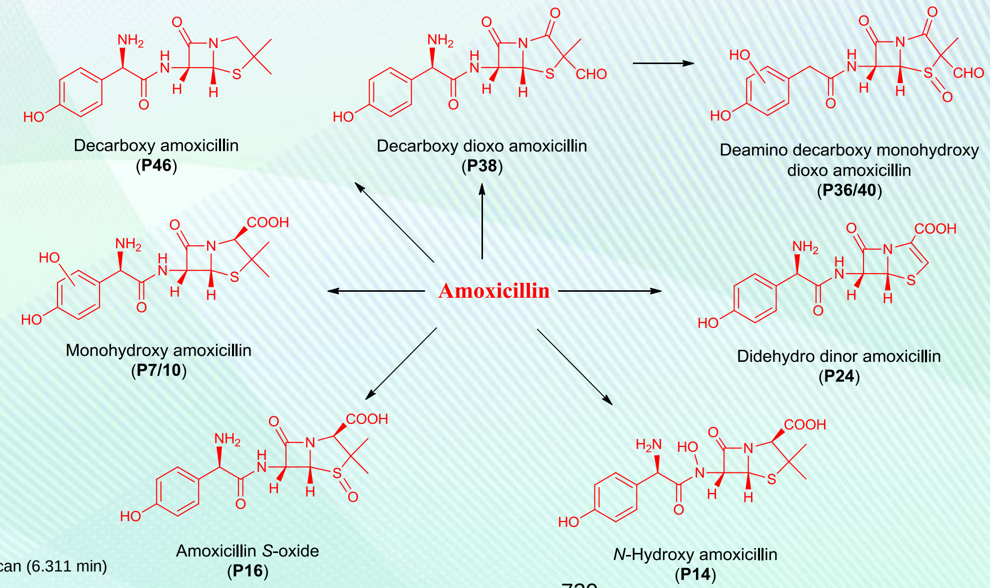
Amoxicillin (AMX)

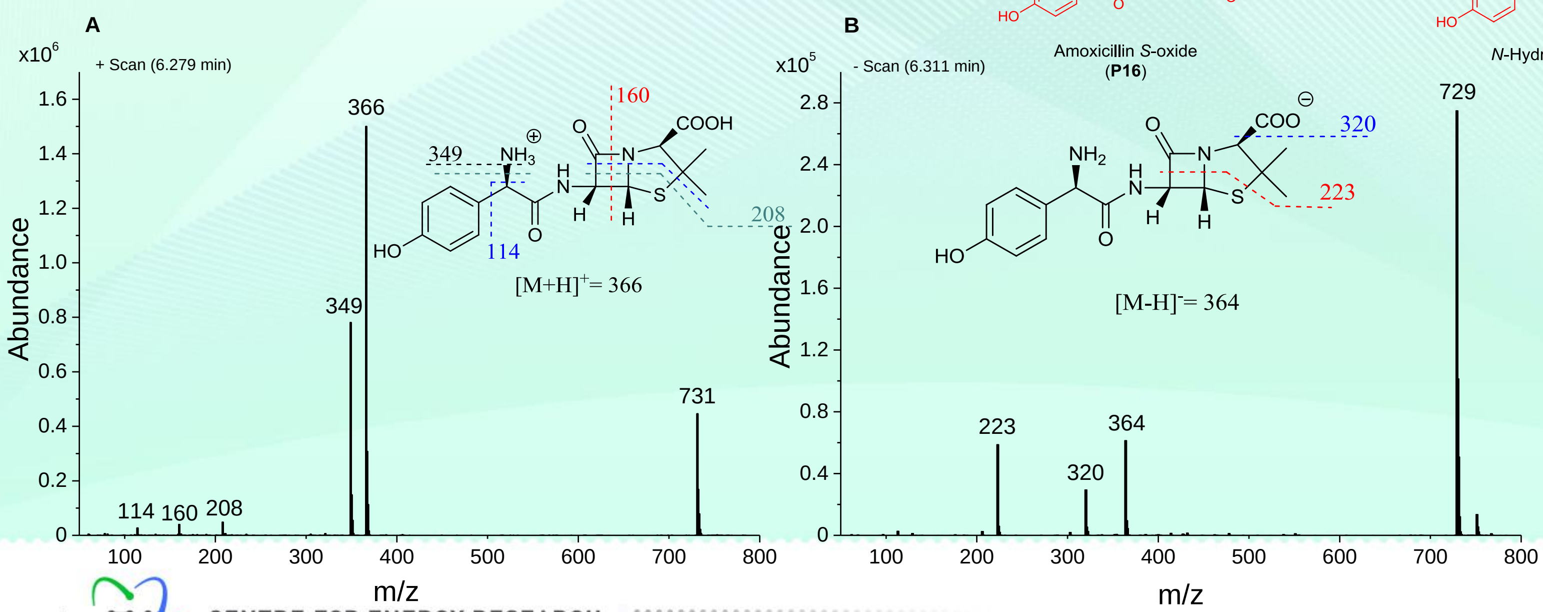
Transient absorption spectra observed of 0.1 mM amoxicillin solution saturated with N_2O (A), and in the same solution containing 0.1 mM $K_3Fe(CN)_6$.

Mechanism of free-radical induced oxidation of AMX LC-MS/MS



Total ion chromatogram





Mass spectra of AMX in positive mode (A) and in negative mode (B)

Mechanism of free-radical induced oxidation

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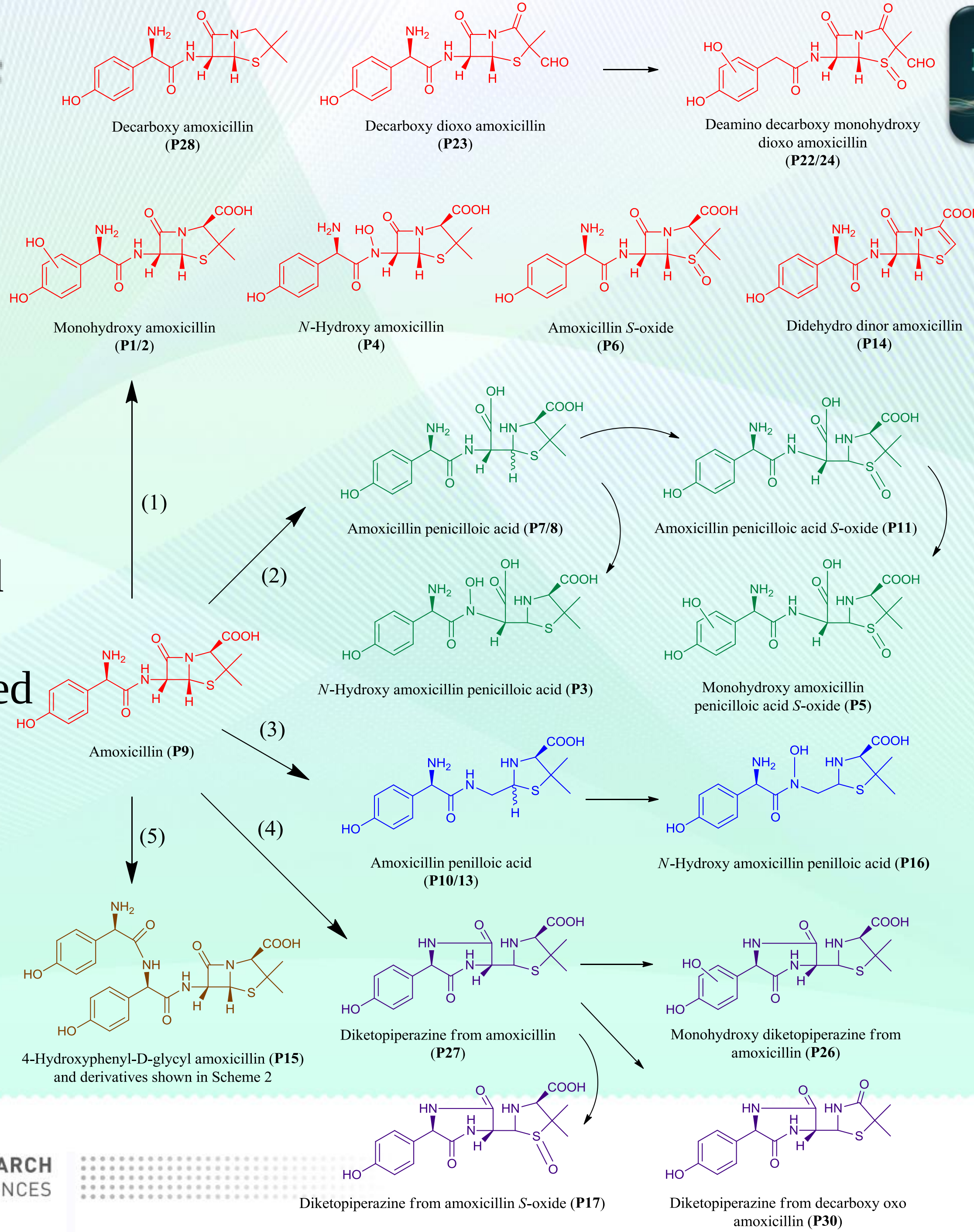
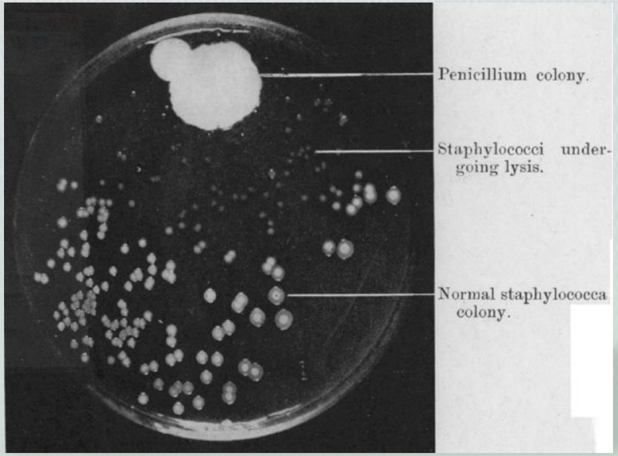
Final products of amoxicillin (**P9**) exposed to oxidative stress. Structures suggested based on LC-MS/MS analysis





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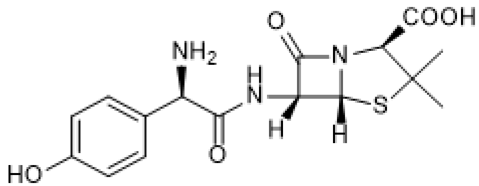
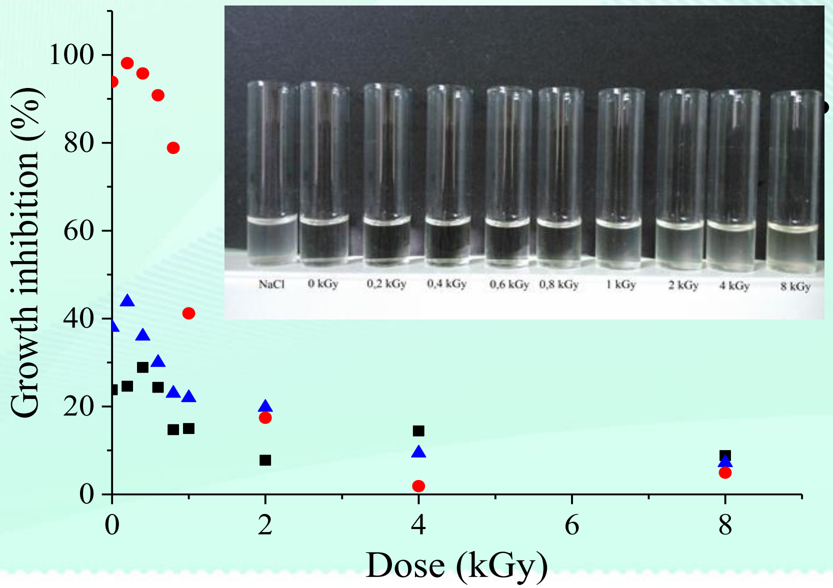
Elimination of the toxicity and antibacterial activity

Elimination of the antibacterial activity

Tracking the elimination of the pharmacophore



Amoxicillin
(AMX)



Microbiology

- Biological assays
  - Acute and chronic effects toward bacteria
    - *Vibrio fischeri* bioluminescence inhibition assay
    - *Vibrio fischeri* growth inhibition assay
  - Bacterial susceptibility tests
    - Agar diffusion test
      - *Staphylococcus aureus*
      - *Bacillus subtilis*
      - *Eschericia coli*
    - Broth macrodilution test
      - *Staphylococcus aureus*
      - *Eschericia coli*

Growth inhibition of *Vibrio fischeri* in amoxicillin (■), cloxacillin (●) and ampicillin (▲) samples as a function of absorbed dose.

Elimination of the antibacterial activity of penicillin derivative (β-lactam) antibiotics

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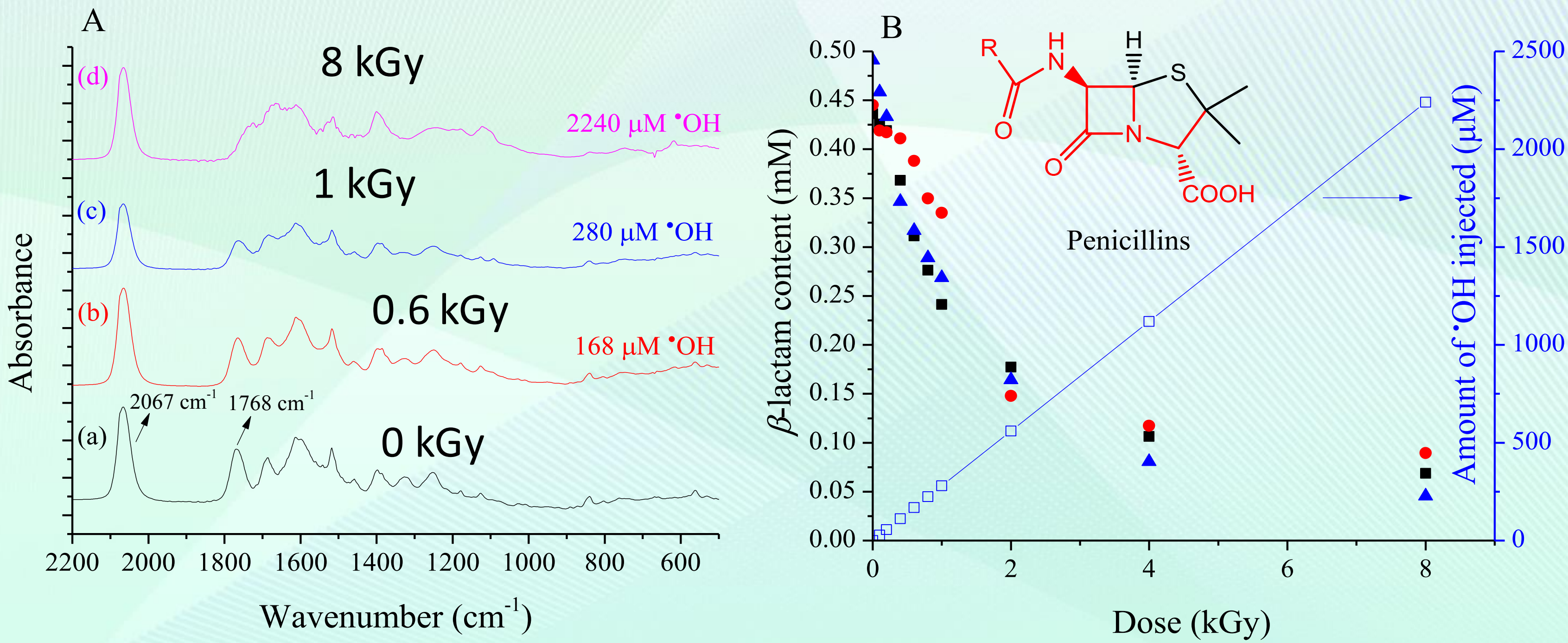
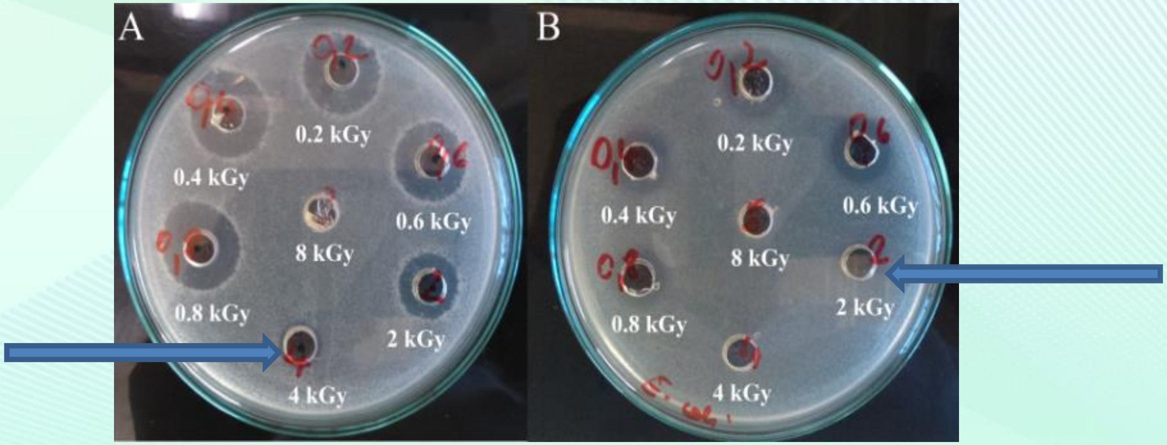


(A) FTIR spectra of amoxicillin, (B) Quantitative FTIR analysis of the *β*-lactam content in the case of amoxicillin (■), cloxacillin (●) and ampicillin (▲) as a function of absorbed dose.

Elimination of the antibacterial activity in purified water

Agar diffusion is a routine method in clinical microbiology to assess bacterial susceptibility to certain antibiotics. It is based on the formation of inhibition zones in the vicinity of the antibiotic solution diffusing in agar media seeded with a certain microorganism.



Agar diffusion test of amoxicillin solutions, treated with increasing dose, on agar plates seeded with *B. subtilis* (A) and *E. coli* (B).

Elimination of the antibacterial activity of erythromycin in a complex synthetic wastewater matrix by EB treatment

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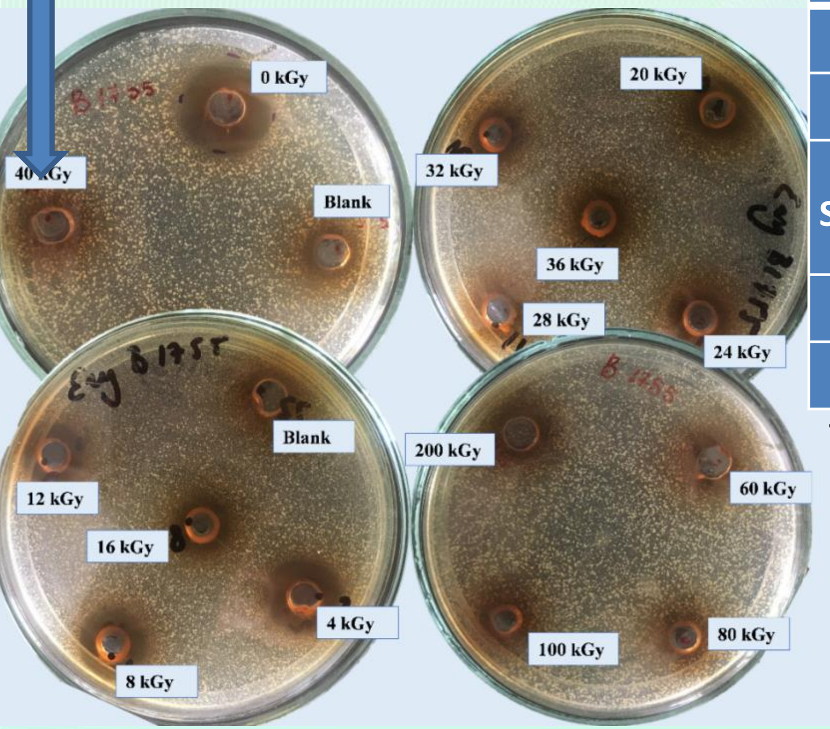
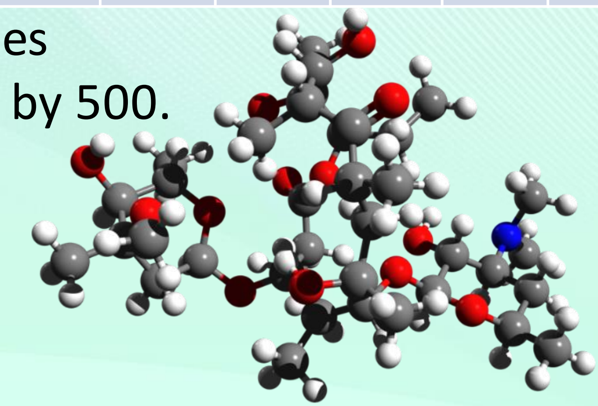
humic acid, inorganic salts



Sample	0 kGy	4 kGy	8 kGy	12 kGy	16 kGy	20 kGy	24 kGy	28 kGy
1.	21.5	16.0	14.0	12.0	12.5	11.5	-	10.0
2.	21.0	16.0	15.0	14.0	12.5	12.0	11.0	-
Sample	32 kGy	36 kGy	40 kGy	60 kGy	80 kGy	100 kGy	200 kGy	
1.	12.0	10.0	-	-	-	-	-	
2.	10.0	10.0	-	-	-	-	-	

The absorbed doses should be divided by 500.

80 Gy



Agar diffusion test using *S. aureus* B.01755 for erythromycin dissolved in the synthetic effluent wastewater matrix received increasing absorbed dose.

SUMMARY

- Based on pulse radiolysis combined with final products analysis degradation mechanism was suggested
- It is important to follow the change in toxicity and in antibacterial activity
- A mild treatment (80 Gy) was just enough to eliminate antimicrobial activity of a complex matrix.

THANK YOU FOR YOUR ATTENTION

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