Óbuda University

PhD Theses

Radiation induced degradation of antibiotics (fluoroquinolones) in aqueous solution

Anna Tegze

Supervisor: Erzsébet Takács, DSc

Óbuda University, Doctoral School on Materials Sciences and Technologies

Centre for Energy Research, Radiation Chemistry Department

Budapest, 2020

I. Background

The release of active pharmaceutical ingredients into natural waters is one of the major problems of our time. Antibiotics are particularly dangerous in this area because they can cause the development of resistant bacteria (Kümmerer, 2001; Baquero, 2001; Dalhoff, 2012). Bacteria that have become resistant in wastewater treatment plants are transferred to natural waters together with the treated wastewater, from which in many cases drinking water is also obtained. Conventional wastewater treatment methods are usually not effective enough for the removal of bioactive ingredients, in some cases more than 90 % of the drug molecules remains in the treated wastewater (Ikehata et al., 2006; Rivera-Utrilla et al., 2013). For this reason, additional treatment is required, to prevent the appearance of pharmaceuticals in surface waters and drinking water. The Advanced Oxidation Processes (AOPs), when used as post-treatment, can be highly effective additional wastewater treatment methods. When using AOP methods, reactive radicals, for example hydroxyl radicals ($^{\bullet}OH$), are formed, which effectively decompose organic contaminants. Ionizing radiation is a less common AOP method. It is environmentally friendly, as it can be used at room temperature and normal atmospheric pressure, it does not require additives, and it is an efficient method even with low energy consumption (Pikaev, 2000; Rivera-Utrilla et al., 2013; He et al., 2016). In addition to the degradation of the drug molecules, irradiation also deactivates bacteria.

Fluoroquinolones are frequently used synthetic antibiotics. Due to their stable structure they are hardly biodegradable (Shah, 1991; King et al., 2000; Andersson and Mac Gowan, 2003). Two second-generation fluoroquinolones were selected in our studies, ciprofloxacin and norfloxacin, as target molecules, as they are widely used antibiotics and they are often detected in treated wastewater and in natural waters. The AOP process is effective when the degradation products have no antibacterial activity, and they are biodegradable.

The ionizing radiation induced degradation of fluoroquinolones has been studied only in a few laboratories. Most publications in this field deal with the determination of rate constants of reactions between fluoroquinolones and reactive radicals using pulse radiolysis.

Only two papers were found on the ionizing radiation induced removal of fluoroquinolones (Cho et al., 2014; Sayed et al, 2016). Cho et al (2014) used high antibiotic concentrations (0.3 mmol dm^{-3}), so only a dose of 10 kGy was effective. This is the only paper where the degradation products of the fluoroquinolones have been identified and their biological effects have been studied (Cho et al., 2014). Hence, there is a lack of extensive studies in the literature on the ionizing radiation degradation of fluoroquinolones, on the degradation products formed during the process and on their biological effects.

II. Objectives

My goal was to determine the removal efficiency of the radiation induced degradation of two fluoroquinolones, ciprofloxacin and norfloxacin and to identify their degradation products, which may help to develop efficient water treatment procedures in the future. Complex analytical studies were performed in aqueous solutions. The initial steps of the irradiation induced decomposition reaction - on the μs time scale - were examined by pulse radiolysis. Larger organic degradation products were isolated and identified by high-performance liquid chromatography and mass spectrometry (LC-MS). The removal efficiency of fluoroquinolones was also examined by LC-MS. Mineralization was monitored by measuring the change in total organic carbon content values, and the degree of oxidation was followed by detecting the change in chemical oxygen demand values. Furthermore, we monitored whether the nitrogen content of the samples changed as a result of irradiation.

In addition to examining the mechanism of degradation and the removal efficiency, we paid close attention to the effects of initial compounds and degradation products on biological systems. There are few and contradictory assumptions about the antibacterial activity of the degradation products of fluoroquinolone compounds. To assess the potential environmental effects, it may be helpful to determine the extent to which the formed degradation products are toxic and badly biodegradable. The concentration of formed H_2O_2 was also determined, as the presence of this compound can greatly affect the biological measurements.

The antibacterial activity was determined by the turbidimetric method using the bacterial strain *Staphylococcus aureus,* while acute toxicity was monitored by *Vibrio fischeri* luminescence inhibition test. Biodegradability was determined from the ratio of biological oxygen demand and chemical oxygen demand values.

III. Experimental methods

The initial concentration of the aqueous solution of ciprofloxacin and norfloxacin samples was adjusted to 0.1 mmol dm^{-3}. Air-saturated solutions were used in most experiments, but in some cases different radical scavengers were applied to examine the reactions of the reactive radicals separately.

For the biological studies and LC-MS measurements, the samples were irradiated using a semi-industrial, panoramic type ^{60}Co gamma radiation source operated by Izotóp Intézet Ltd. Short-lived intermediates (radicals) were studied with pulse radiolysis equipment. This is a TESLA LINAC LPR-4 electron accelerator connected with a kinetic spectrophotometer (UV-Vis) detector. Reaction rate constants were determined directly from the kinetic curves obtained during pulse radiolysis.

Fluoroquinolones and their degradation products were separated and determined by LC-MS/MS (Agilent 1200 LC and Agilent 6410 MS, EVO C18 100A New Column stationary phase). Gradient elution was used for the separations. Using this method and values obtained by chemical oxygen demand and total organic carbon content measurements, the removal efficiency of fluoroquinolones was determined. Chemical oxygen demand (COD) measurements were performed according to the MSZ ISO 6060:1991 standard, in sulfuric acid medium and at high temperatures. A Shimadzu TOC-L CSH/CSN instrument was used to measure total organic carbon (TOC) and total nitrogen (TN) contents.

H_2O_2 formed during radiolysis of water can interfere with biological studies, so we monitored the change in H_2O_2 concentration using the Cu(II)/phenanthroline test. Biological oxygen demand (BOD) measurements were performed according to DIN EN 1899 standard with activated sludge inoculation. The OxiTop® Control system was used for the measurements. The evaluation was based on 10-day BOD values. In the toxicity study, the natural light emission of *Vibrio fischeri* bacteria was measured. Acute toxicity was determined by the Microtox® luminescence inhibition test according to DIN EN ISO 11348-3 standard using a HACH – LANGE Gmbh LUMIStox 300 luminometer. Antibacterial activity was measured on *Staphylococcus aureus* bacterial strains for 24 h by turbidimetric method with a Multiskan Ascent optical densitometer.

IV. New scientific results, thesis points

1. Ionizing radiation is an effective method to remove ciprofloxacin and norfloxacin from aqueous solutions. A dose of 2 kGy is sufficient to remove the initial compounds from 0.1 mmol dm^{-3} solutions, but the removal of the initial compounds was not accompanied by a significant degree of oxidation and mineralization. The initial compounds are first step-by-step oxidized and finally mineralized. Fluoroquinolones are very stable compounds. A dose of 6 kGy is required for oxidation to ∼50 % and mineralization to ∼40 %. *(Publication 1)*

2. Ciprofloxacin and norfloxacin react with hydroxyl radicals ($^•OH$) and hydrated electrons (e_{aq}^-). In the reaction with $^•OH$ hydroxycyclohexadienyl type radicals are formed, in the reaction with e_{aq}^- the electron adducts of ciprofloxacin and norfloxacin are produced. Hydroxycyclohexadienyl type radicals may have strong absorption bands between 300 and 400 nm. These absorption bands cannot be studied due to the strong absorption of the initial compounds below 400 nm. However, the absorbances of these strongly conjugated radicals and their transformation products are also visible in the range above 400 nm. The electron adducts of ciprofloxacin and norfloxacin have absorption bands around 600 nm. At these absorption bands, a rapid decay is observed on a 10 μs time scale as the adducts are rapidly protonated and cyclohexadienyl type radicals are formed. *(Publication 2)*

3. The main degradation products of ciprofloxacin and norfloxacin are hydroxylated variations of the starting compounds, desethylene–ciprofloxacin and –norfloxacin, defluorinated products and anthranilic acid analogues of ciprofloxacin and norfloxacin. Fluoroquinolones and their metabolites were separated and identified by LC-MS/MS. For the analysis samples irradiated with a dose of 0.5 kGy were used, because at this dose mostly higher molecular weight, aromatic degradation products detectable by LC-MS/MS were in the solutions. *(Publication 2)*

4. Significant amount of H_2O_2 is generated during the irradiation of dilute aqueous solutions. It was found that the easily oxidizable degradation products formed interfere with the Cu(II)/phenanthroline test. This should be taken into account when applying the test. *(Publication 2 and 3)*

5. Irradiation significantly increases the biodegradability of ciprofloxacin and norfloxacin in solutions, making them accessible to microorganisms of activated sludge. The initial compounds and the first degradation products are not biodegradable. This is also supported by the product analysis, as the structure of the products formed at lower doses does not differ much from the initial compounds. *(Publication 1 and 2)*

6. Some of the formed degradation products of ciprofloxacin and norfloxacin are more toxic than the parent compounds, the toxicity was reduced at higher doses. The increase in toxicity may be explained by the formation of low molecular mass organic acids, which are reported to be toxic to the *V. fischeri* test bacterium. *(Publication 1)*

7. The degradation products of ciprofloxacin and norfloxacin have no antibiotic effect. This is supported by the structure of the degradation products and by antibacterial activity studies. Based on the structure of the degradation products, it can be assumed that many products have no antibacterial effect due to the cleavage of the carboxyl and keto groups on the active part of the compounds. Other products may have reduced antibacterial activity due to the cleavage or loss of the piperazinyl ring or loss of the fluorine atom. The strong correlation between the decrease in initial compound concentration and antibacterial activity suggests that the degradation products have no antibiotic activity. *(Publication 1 and 2)*

V. Utilization possibilities of the results

Both a detailed description of the general degradation mechanism and an accurate knowledge of the degradation efficiency may contribute to the optimization of water treatment processes based on ionizing radiation in the future. The results of biological studies can help to estimate the potential environmental impacts of degradation products released into the environment.

Based on the above results, ionizing radiation is an effective method to remove fluoroquinolones from aqueous solution, and it would be worthwhile to supplement conventional wastewater treatment with irradiation post-treatment. Such a procedure has been used on a laboratory or semi-industrial scale in many countries around the world (e.g., Brazil, China, Korea, USA), and on an industrial scale, a plant for textile wastewater treatment was put into operation in China this year. Scientists working in the field of radiation chemistry served as advisers during the installation of the industrial facility. If a decision is made to introduce the method in Hungary, the results of our laboratory, including my work, can be utilized.

VI. References

Andersson, M. I., MacGowan, A. P., 2003. Development of the quinolones. Journal of Antimicrobial Chemotherapy 51, 1–11.

Baquero, F., 2001. Low-level antibacterial resistance: a gateway to clinical resistance. Drug Resistance Updates 4, 93–105.

Cho, J.Y., Chung, B. Y., Lee, K., Lee, G., Hwang, S.A., 2014. Decomposition reaction of the veterinary antibiotic ciprofloxacin using electron ionizing energy. Chemosphere 117, 158–163.

Dalhoff, A., 2012. Global Fluoroquinolone Resistance Epidemiology and Implications for Clinical Use. Interdisciplinary Perspectives on Infectious Diseases, 2012, ID 976273

He, S., Sun, W., Wang, J., Chen, L., Zhang, Y., Yu, J., 2016. Enhancement of biodegradability of real textile and dyeing wastewater by electron beam irradiation, Radiation Physics and Chemistry 124, 203–207.

Ikehata, K., Naghashkar, N. J., El-Din, M. G, 2006. Degradation of Aqueous Pharmaceuticals by Ozonation and Advanced Oxidation Processes: A Review. Ozone: Science & Engineering 28, 353–414.

King, D. E., Malone, R., Lilley, S. H., 2000. New classification and update on the quinolone antibiotics. American Family Physician 61., 2741–2748.

Kümmerer, K., 2001. Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. Chemosphere 45, 957–969.

Pikaev, A.K., 2000. Current status of the application of ionizing radiation to environmental protection: II. Wastewater and other liquid wastes (a review). High Energy Chemistry 34, 55–73.

Rivera-Utrilla, J., Sánchez-Polo, M., Ferro-García, M.Á., Gonzalo Prados-Joya, G., Ocampo-Pérez, R., 2013. Pharmaceuticals as emerging contaminants and their removal from water. A review. Chemosphere 93, 1268–1287.

Sayed, M., Khan, R.A., Khan, A.R., Khan, A.M., Shah, L.A., Khan, J.A., Shah, N.S., Khan, H.M., 2016. Hydroxyl radical based degradation of ciprofloxacin in aqueous solution. Journal of the Chilean Chemical Society 61, 2949–2953.

Shah, P. M. 1991. Ciprofloxacin–review. International Journal of Antimicrobial Agents 1, 75–96.

VII. Publications related to theses

1.) A. Tegze, G. Sági, K. Kovács, R. Homlok, T. Tóth, C. Mohácsi-Farkas, L. Wojnárovits, E. Takács 2018. Degradation of fluoroquinolone antibiotics during ionizing radiation treatment and assessment of antibacterial activity, toxicity and biodegradability of the products. Radiation Physics and Chemistry 147, 101-105.

IF: 1,9 References: 17 (independent: 16)

2.) A. Tegze, G. Sági, K. Kovács, T. Tóth, E. Takács, L. Wojnárovits 2019. Radiation induced degradation of ciprofloxacin and norfloxacin: Kinetics and product analysis. Radiation Physics and Chemistry 158, 68-75.

IF: 2,23 References: 5 (independent: 5)

3.) E. Illés, **A. Tegze**, K. Kovács, Gy. Sági, Z. Pap, E. Takács, L. Wojnárovits 2017. Hydrogen peroxide formation during radiolysis of aerated aqueous solutions of organic molecules; Radiation Physics and Chemistry 134, 8-13.

IF: 1,45 References: 11 (independent: 6)

VIII. Further publications

4.) R. E. Abutbul, E. Segev, U. Argaman, **A. Tegze**, G. Makov, Y. Golan 2019. Stability of cubic tin sulphide nanocrystals: role of ammonium chloride surfactant headgroups. Nanoscale, 11, 17104–17110.

IF: 6,97 References: 2 (independent: 0)

Conference papers:

5.) Tegze A., Sági G., Takács E., Wojnárovits L. 2018. Norfloxacin vizes oldatának lebontása ionizáló sugárzással: termékanalízis és kinetikai vizsgálatok. Őszi Radiokémiai Napok 2018. 55-59., ISBN 978-963-9970-93-9

6.) Tegze A., Sági G., Takács E., Wojnárovits L. 2017. Norfloxacin vizes oldatának lebontása gamma sugárzással, valamint a bomlástermékek biológiai vizsgálata. Őszi Radiokémiai Napok 2017. 22-27., ISBN 978-963-9970-80-9

7.) Tegze A., Illés E, Takács E. 2016. Ciprofloxacin és norfloxacin nagyenergiájú ionizáló sugárzással indukált bontása híg vizes oldatokban. Őszi Radiokémiai Napok 2016. 39-42., ISBN 978-963-9970-69-4

8.) Tegze A. 2016. Ciprofloxacin és norfloxacin gamma sugárzással indukált lebontása Műszaki Kémiai Napok 2016, 124-128., ISBN 978-963-396-087-5