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Review of the doctoral dissertation of M.Sc. **Stephen Ochieng Kabasa**, entitled

Radiation-induced Degradation of Selected Chlorinated Emerging Organic Pollutants in Aqueous Solution under Electron Beam Irradiation

submitted to the scientific committee of the Institute of Nuclear Chemistry and Technology for the award of Ph.D. degree in Natural Sciences in the discipline of Chemical Sciences.

Supervisor: D.Sc. **Yongxia Sun**, Professor ICHTJ

The main goal of the doctoral thesis was to investigate the degradation of chloroquine (CQ) and hydroxychloroquine (HCQ) in aqueous solutions induced by ionizing radiation.

CQ and HCQ are quinoline derivatives and are synthetic substitutes for quinine introduced to medicine: CQ in 1934, HCQ in1946 for its antimalarial activity. Later, these pharmaceuticals began to be used in the treatment of rheumatoid arthritis, cancer therapy, arthritis and most recently CQ was proposed for treatment of SARS-Covid 19. Therapeutic administration of these drugs releases CQ, HCQ and their metabolites into wastewater. Studying CQ and HCQ degradation processes is important from the point of view of medical wastewater treatment, but also provides knowledge on methods of decontamination of the entire family of halogenated organic pollutants.

The doctoral dissertation has 187 pages (the last page is blank), including 12 appendices (pp. 171-186). On page seventeen of the dissertation, the five most important goals are presented. Three of them are particularly important, i.e. examining the impact of various parameters (dose, dose rate, solution pH, presence of scavengers, etc.) on the efficiency of CQ and HCQ degradation. Another issue is the identification of CQ and HCQ degradation products after EB irradiation and the study of their properties, including the analysis of their toxicity. The culmination of the thesis is a numerical simulation of the degradation of CQ and HCQ in order to develop the degradation mechanism and confront it with the experimental results.

The dissertation is divided into five chapters. I read Chapters 1 and 2 with interest, especially those parts regarding the technology for removing emerging pollutants. The Author made a detailed review of the literature on the subject, describing various methods, including: electrochemical and chemical oxidation, membrane technologies, biological methods, catalysis (photolysis/photocatalysis, Fenton processes) and others.

I consider this dissertation to cover technical problems. In the broad sense, it is dedicated to specialists addressing the problem of degradation of water pollutants by organic compounds. It is based on three journal papers. I would like to emphasize that the dissertation contains many very interesting and new results, presented in a good and simple way. The length of the chapters is properly balanced. The quality of figures, tables and diagrams is high. Overall, the very well-prepared text contains some editorial errors. For example:

- the hydroxyl radical symbol appears over 260 times, but it is used inconsistently and only about forty times in the generally accepted superscript dot manner. The symbol of the hydrated electron was treated more uniformly in the work, although even in this case editorial errors can be found;

- equations 4.1, 4.3 are written incorrectly: page 79 (the radical symbol is missing);

- the title of the dissertation in Polish is a mistranslation of the original title. I am particularly surprised by the appearance of the term "newly discovered" in the title of a PhD thesis in Polish.

The Author did not provide a definition of ‘removal efficiency’ in the text dissertation. I assume that for this purpose he used measurements of changes in the absorbance of a solution, e.g. HCQ (Figure 4.1b) as a function of the dose absorbed by the tested system. I am very interested in how the absorbance of products formed in the radiolytic process was taken into account in the spectral analysis of HCQ degradation. Is there a correlation between the calculated "removal efficiency" value based on the decrease in substrate absorbance and that analyzed by the increase in product absorbance, e.g. at 370 nm, as a function of dose?

The influence of pH on conducting experiments is one of the key issues used by the Author to explain the radiative degradation of the tested pollutants. Unfortunately, it is very difficult for the reader to find information on how the pH of the tested solutions was controlled. An inquisitive reader will find it in the publication co-authored by the PhD student [1] that: *The pH values were attained by adjusting the pH of the solution with 0.1 M NaOH and 0.1 M sulfuric acid.* In another publication [2] we find: *All experiments were performed in ambient conditions (STP) without adjusting pH unless otherwise stated.* I read this work carefully and unfortunately I did not find a reference to "unless otherwise stated."

In my opinion, the information regarding the technical parameters of the ILU6 accelerator is incomplete. In general, Chapter 3.4.1 is prepared very carelessly and superficially. The listed technical parameters of the accelerator are not described with details. Of course, experts will know what 1.6 MeV, 2 Hz and 50 mA mean. Figure 3.2 does not show the experimental system for irradiation of the tested aqueous solutions with the EB of the ILU6 accelerator. The correct figure is Fig. 3.3. This drawing shows a different energy value of the electron beam, namely: 1.65 MeV. Which value, 1.6 or 1.65 MeV, is correct? In the absence of a detailed description of Figure 3.3, it is difficult to understand why *'the sample thickness is estimated to be 1.5 mm'*? If schematically, the blue rectangle in the figure represents the 'top view' of the sleeve bag and has dimensions of 80 × 170 mm and contains 35 ml of solution, and its thickness should be 2.57 mm, not 1.5 mm, as stated in the text of the dissertation. I am interested in what the doses in Figure 3-3, 14.7 kGy and 20 kGy, represent?

There is clearly no information about the duration of the electron pulse of the accelerator used. What was the pulse repetition range available, was it only 2 Hz? I know from my own experience that in technological processes, the frequency of pulses is extremely important for the efficiency of the process. This applies to many areas of radiation processing, from hardening drills to obtaining biomaterials made from synthetic and natural polymers. I am very interested in the Author's opinion on how increasing the repeatability of electron beam pulses to 50 Hz should change the decomposition process of the tested compounds?

The use of high concentrations of HCQ and CQ (around 125 mg/L) in spectroscopic measurements may cause significant errors in absorbance registration. Figure 4.1 shows the spectra of aqueous solutions of CQ and HCQ, for which the maximum absorbance reaches a value of 5.7. Using the molar absorbance coefficient of HCQ at pH 6 (Rath 2023), which is 17610 $dm^3\ mol^{-1}\ s^{-1}$, the maximum absorbance value in Figure 4.1 B should be 5.07 instead of 5.7! Please explain how such high absorbance values were measured using the Jasco V670 spectrophotometer? Unfortunately, I did not find any information on the use of cuvettes with an optical path smaller than 1 cm or special attenuators either in the dissertation or in publications related to it. The Author did not determine the molar absorbance coefficients of CQ and HCQ. This is a serious error because it is known that the intensity and shape of the UV spectrum of HCQ depend on the pH value of the solution. Even more serious error is the lack of information about at what pH value the degradation process of CQ and HCQ took place, as illustrated in Figures 4.1, 4.7, 4.9. On page 126 there is section 4.8.1 *Changes in pH.* It contains contradictory information regarding pH changes during radiolytic degradation of the tested compounds. The Chapter contains one figure, namely Figure 4-22, but it is not mentioned in the text! We can read in the thesis: *The pH of the aqueous solutions dropped from 6 to 3.8 with increasing irradiation and reducing CQ and HCQ concentration*, and the figure shows that the initial pH was 7. Please explain what the actual value is?

Analysis of data from publication [Rath et al., 2023] Figure 2a allows one to conclude that the value of the reaction constant of hydrated electron with HCQ is greater than 10^{10} dm^3 $mol^{-1}\ s^{-1}$ rather than equal to $2\times10^9\ dm^3\ mol^{-1}\ s^{-1}$. I want to clearly emphasize that I do not criticize the authors of the paper [Rath et al., 2023] and I do not question the sense of including this data ($k=2\times10^{9}$ dm^{3} mol^{-1} s^{-1}) in the doctorate. The hydrated electron reacts with nitroquinolines (namely 3-NQ, 5-NQ, 6-NQ, 7-NQ, 8-NQ) with rate $(4.0\pm0.2)\times10^{10}$ dm^{3} mol^{-1} s^{-1}[3]. Primaquine is reduced by the e_{aq}^{-} at neutral pH with a rate constant of $(2.47 \pm 0.1)\times10^{10}$ dm^{3} mol^{-1} s^{-1} [4], amodiaquine reacts with e_{aq}^{-} with rate constant $(3.9\pm0.1)\times10^{10}$ dm^{3} mol^{-1} s^{-1} [5]. Almost identical value, $(3.8\pm0.3)\times 10^{10}$ dm^{3} mol^{-1} s^{-1}, was obtained for mefloquine, and is typical of a diffusion-controlled reaction [6]. The electron reaction constant with hydroxyquinoline reaches a similar value: 3.0×10^{10} dm^{3} mol^{-1} s^{-1} [7]. In my opinion, such a large differences in the values of the rate constants of scavenging of e_{aq}^{-} by HCQ in relation to other quinoline derivatives requires explanation. The first step to achieve this this goal should be to measure the rate constant of e_{aq}^{-} reaction with HCQ using the pulse radiolysis system located at the Institute of Nuclear Chemistry and Technology. I think, based on my results of pulse radiolysis measurements of e_{aq}^{-} reactions with compounds containing isoquinoline or quinoline moieties, that the scavenging of e_{aq}^{-} by HCQ will be a diffusion-controlled reaction. If, in fact, the reaction is more than 10 times slower than the diffusion-controlled reaction, it should provide a reason to prepare a separate publication to explain this phenomenon. The electron transfer rate constant is described by Marcus' theory and depends, among other factors, mainly on the driving force of reaction (free enthalpy of the reaction). Assuming the reduction potentials for CQ and HCQ are the same, please suggest what other factors could so significantly (tenfold) affect the effectiveness of the scavengers used?

A brief comment on Table 4.1:

Table 4.1 lacks data regarding the reaction of hydrated electron with quinoline. The value of this constant is 7.1×10^{9} dm^{3} mol^{-1} s^{-1} . [8]

The value of bimolecular rate constant for the reaction between chloroquine (pH 7.0) with $^{\bullet}$OH radical has been well known since 1980s and is $(5.4\pm0.1)\times10^{9}$ dm^{3} mol^{-1} s^{-1} [9].

I would like to acknowledge that the doctoral dissertation contains the results of measurements using liquid chromatography/mass spectrometry to identify CQ and HCQ degradation products. Oxamic and oxalic acid are most likely responsible for the decrease in the pH of CQ and HCQ solutions after irradiation. Was the PhD student able to identify these acids using LC-MS spectrometry? The HCQ molecule is characterized by electrochemical (irreversible) oxidation with the transfer of two electrons coupled with the transfer of two protons. A product is obtained with a molecular weight two units higher than HCQ. Was it possible to identify the m/z 338 peak among the recorded LC-MS spectra after radiolysis of HCQ solutions? During radiolysis of CQ and HCQ solutions studied as part of the doctoral dissertation, both oxidation and reduction processes occur simultaneously or selectively through the use of appropriate scavengers for specific reactive species. In my opinion, among the scavengers used, there are too few of those that lead to the selective oxidation of the pollutants tested. Probably the only oxidant used was the $SO_4^{\bullet-}$ radical. The author rightly draws attention to the complexity of CQ or HCQ degradation processes induced by the hydroxyl radical. Comparison of the degradation efficiency of CQ and HCQ induced by oxidative radicals such as $N_3^{\bullet}$, Cl_2^{-}, Br_2^{-}, $SO_4^{-\bullet}$ with that generated by the hydroxyl radical should be helpful in elucidating the main channel of $^{\bullet}OH$ action. I would like to make it clear that my proposals to perform additional measurements are not a criticism of the doctoral dissertation. This indicates that a number of issues related to the mechanism of radiation degradation of CQ and HCQ definitely deserve further research. The overall assessment of the dissertation is very positive.

Based on the Scopus database, I conclude that M.Sc. Kabasa is the co-author of four scientific publications in peer-reviewed journals. The Hirsh index is 1, but this is due to the fact that the publications were published recently. Information about the last one appeared on the internet on January 5, 2025. The total impact factor of the publication is 4.9.

I believe that this doctoral dissertation shows that Mr. Stephen Ochieng Kabasa has demonstrated the ability to conduct independent scientific research. I have no doubt that the findings contained in this dissertation make a valuable contribution to the development, in particular, of radiation technology for wastewater treatment, as well as radiation chemistry in general.

Concluding, the thesis prepared by M.Sc. Stephen Ochieng Kabasa: (1) contains original and valuable scientific results that are adequately discussed, (2) concerns a very attractive topic in modern radiation chemistry, with potential applications of the electron beam in the process of purifying polluted water, (3) was published in three peer-reviewed journals of which the Ph.D. student was listed as the first author, (4) it was written precisely and edited quite carefully. It meets the requirements for receiving the PhD degree in Chemical Sciences. In my opinion, M.Sc. Stephen Kabasa's doctoral dissertation meets the formal and customary requirements for doctoral theses. In connection with the above, I am submitting a formal application to the Scientific Committee of the Institute of Nuclear Chemistry and Technology to admit Mr. Kabasa to the next stages of the doctoral process.

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