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Degradation Of Ciprofloxacin By Electrochemical Advanced Oxidation Process Using C/Pbo$_2$ Anode

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Abstract – The occurrence of pharmaceutical substances in wastewater represents a significant environmental issue, highlighting the urgent need for their degradation to safeguard ecological integrity. This study investigates the electrochemical advanced oxidation process (EAOP) for the degradation of ciprofloxacin (CIP), utilizing a lead dioxide-coated graphite (C/PbO$_2$) anode. The anode was produced by applying a lead dioxide layer onto an inert graphite electrode. Under conditions of a current density of 30 mA cm^{-2}, a pH of 3 (±0.5), and an electrode spacing of 2 cm, the degradation of CIP, initially at a concentration of 1 mg L^{-1}, reached 98% within 30 minutes. The degradation process followed a pseudo-first-order kinetic model. The predominant mechanism for degradation was identified as the action of hydroxyl radicals, confirmed by radical quenching tests using t-butanol. Additionally, a total organic carbon (TOC) removal rate of 85% demonstrated that CIP was not only converted into byproducts but also effectively mineralized. Toxicity assessments conducted on the reaction intermediates and byproducts against E. coli revealed lower toxicity levels compared to the original CIP influent.

***Keywords*:** Electrochemical advanced oxidation process; antibiotics; CIP; toxicity; byproducts; mineralization.

1. Introduction

As medicines were evolving day by day and increasing depending upon the treatment technologies, the misuse and overuse of these antibiotics have emerged as a global concern. The residual antibiotic concentration from human excretion was mixed up with water bodies, subsequently developing antimicrobial resistance (AMR) [1]. Study on the presence of antibiotics in surface water, wastewater, groundwater, and even drinking water was started in the 1970s. Since then, various studies have been carried out in different countries, including Canada [2], USA [3], and China [4]. Recently, in India, some reports have indicated the presence of different antibiotics in hospitals and municipal wastewater. These results have been reported from Patancheru, Ujjain, and Delhi regions [5], [6]. A report by Mutiyar and Mittal (2014) reflects the high concentrations of 21 antibiotic compounds, which were more than 1 µg L^{-1}. Whereas for 11 compounds, the concentration was above 100 µg L^{-1}. This was the highest reported concentration of antibiotics in any wastewater. The concentration of ciprofloxacin, centizine, enrofloxacin, norfloxacin, lomefloxacin, and enoxacin was 28000-31000, 1300-1400, 780-900, 390-420, 150- 300, 150-300 µg L^{-1} respectively [7].

Ciprofloxacin (1-cyclopropyl-6–fluoro-1,4–dihydro–4–oxo–7-(1-piperazinyl)–3-quinoline carboxylic acid) (CIP), is one of the antibiotics of quinolone group which was used in a wide spectrum throughout the world for generally bacterial infections has been selected as a target material for degradation in this study. It belongs to the fluoroquinolone groups of antibiotics, and the chemical structure is shown in Fig. 1 [8]. The conventional treatment processes used in wastewater treatment plants [9], [10], [11] were not favorable enough for separating the antibiotics from the wastewater matrix. One of the most efficient processes for the degradation of emerging contaminants is the advanced oxidation process (AOP) due to the generation of hydroxyl radicals (OH$^\bullet$), which has a standard oxidation-reduction potential of 2.8 V, which is only lower than that of fluorine ($E^o = 3V$ vs. SHE) [12]. The high reactivity of OH$^\bullet$ towards the organic contaminant and the ability to mineralize the cyclic and long-chain organics to simple end products made it one of the most advantageous oxidizing agents for the degradation of persistent organic pollutants. Unlike various AOPs such as ozone-based processes [13], photolysis, photocatalysis processes [14], and Fenton reaction-based processes [15], electrochemical advanced oxidation processes (EAOPs) have received growing attention due to their high-energy efficiency, low chemical consumption, and versatility [16]. Here, OH$^\bullet$ is generated by applying an external current over the water matrix [17].

The primary limitations associated with this specific technology encompass both operational expenses and the requirements for electrical supply. Additionally, there is a potential for the generation of byproducts that may exhibit greater bio-recalcitrance and/or toxicity compared to the original pollutants [18]. The efficacy of the electrodes is compromised by a decline in their activity and a reduction in lifespan due to fouling, which involves the accumulation of organic materials on their surfaces [19]. A variety of electrodes, distinguished by their diverse types and material properties, including BDD, SnO_2, IrO_2, PbO_2, have been employed to date [20], [21], [22]. Lead dioxide electrodes can be used as non-reactive anodes, which can efficiently degrade pharmaceuticals and other emerging pollutants from the water using EAOP [23]. It was also observed that the EAOP is mostly a surface phenomenon where the main governing criterion is the surface area. To mitigate lead consumption, a graphite substrate lead dioxide electrode (GSLD) has been developed, demonstrating significant mechanical strength and favorable conductivity.[24].

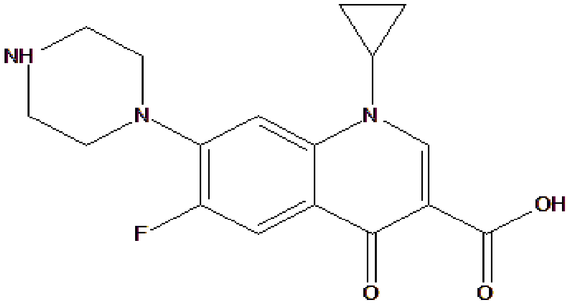


Fig. 1: Chemical structure of CIP

A significant number of studies have been conducted on the removal of antibiotics through EAOP utilizing lead dioxide anodes. These studies have primarily concentrated on optimizing reaction conditions, elucidating oxidation kinetics, and evaluating overall process efficiency. However, the formation of byproducts and their toxicity assessment are vital considerations within the framework of environmental water matrices. Consequently, thorough research into these byproducts and their toxicological implications is essential for environmental protection.

The current investigation aims to analyze the efficiency, reaction kinetics, and degradation mechanisms of CIP through the EAOP method employing a lead dioxide-coated graphite (C/PbO_2) anode. Additionally, this study seeks to evaluate the mineralization efficiency and the residual toxicity of the treated wastewater.

2. Materials and methods

2.1. Chemicals and reagents

Pure CIP powder was obtained from Alfa Aesar (Haverhill, Massachusetts, United States). A stock solution of CIP having a strength of 100 mg L^{-1} was prepared in deionized water and stored in a round-bottom volumetric flask of 1000 mL at room temperature. Pure HPLC-grade solvents like acetonitrile, water, and acetic acid were brought from Thermo Fisher Scientific (Waltham, Massachusetts, United States). Analytical grade lead nitrate was brought from Qualigens. Copper nitrate, anhydrous sodium hydroxide pellets, anhydrous sodium sulfate, and sodium chloride were obtained from Spectrochem. For dilution and sample preparation, deionized water was used.

2.2. Analytical methods

CIP was analytically detected using HPLC (high-performance liquid chromatography) coupled with a diode array detector (DAD) (model: Dionex Ultimate 3000, Thermo Fisher Scientific). In the case of wastewater samples, it was first filtered through a 13 mm nylon syringe filter having a pore size of 0.2 µm and then separated and quantified in the HPLC column. The analyte was separated on an acclaim C18 column (4.6 mm × 150 mm; 3µm pore size). An isocratic mobile phase containing acetonitrile and 2% acetic acid (25:75 v/v) was used. The flow rate was maintained at 750 µL min-1. The peak was detected at 280 nm UV wavelength at 4.5 minutes. The injection volume was set to 50 µL. The limit of detection (LOD) of the sample was found to be 10.5 µg L^{-1}.

2.3. Degradation of CIP by EAOP in batch mode

The EAOP was carried out inside a complete mixed batch reactor (Fig. 2) with an effective volume of 250 ml. Continuous mixing was done at 500 rpm using a magnetic stirrer. Cylindrical shaped (10 mm Ø) C/PbO_2 electrode was used used as an anode, and stainless steel was used as a cathode. The effective surface area of the anode was calculated to be 35 be 35 cm^2. The distilled water matrix was spiked up initially with 1 mg L^{-1} of CIP. The pH, current density, electrode spacing, and electrolyte (Na_2SO_4) concentration were 3(±0.5), 30 mA cm^{-2}, 2 cm, and 150 mM, respectively. The values for for these parameters were determined based on findings from earlier research on the EAOP of fluoroquinolone antibiotics antibiotics [25][26][27][28]. The degradation experiments were conducted up to 30 min and samples were withdrawn at at regular intervals to determine the residual CIP concentration. The percentage degradation was determined using the following equation (eq.1).

$$\text{Percentage degradation} = \frac{[\text{CIP}]_o - [\text{CIP}]}{[\text{CIP}]_o} \times 100 \tag{1}$$

Where, $[\text{CIP}]_o$ is the initial CIP concentration and $[\text{CIP}]$ is the concentration at any time t.

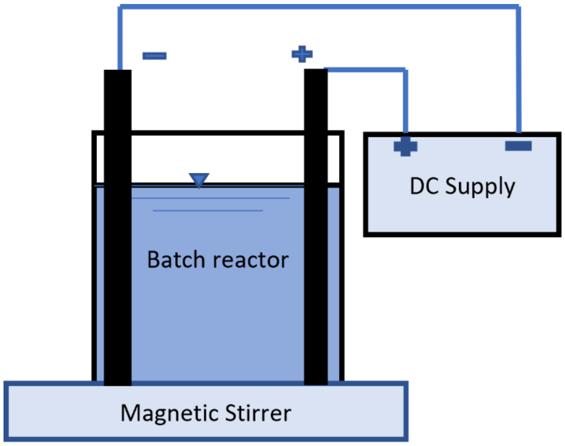


Fig. 2: Schematic diagram of laboratory scale batch reactor for EAOP of CIP

Kinetic analysis was made considering a pseudo-first-order kinetic model and the integrated form of the first-order model is shown below eq. (2).

$$\ln \frac{[CIP]}{[CIP]_o} = -kt \tag{2}$$

Where, k is the apparent pseudo-first-order reaction rate constant.

The mineralization efficiency was analyzed by measuring the initial and residual TOC of the sample. The TOC was measured using a Shimadzu TOC-L analyzer with an autosampler tray. Samples were introduced inside 30 ml screw-capped airtight vials in the autosampler. The mineralization efficiency (ME) and current efficiency (CE) were calculated by the following equations (eq. (3) and (4)).

$$\text{ME}\ (\%) = \frac{(\text{TOC}_\text{i} - \text{TOC}_\text{f})}{\text{TOC}_\text{i}} \times 100 \tag{3}$$

$$\text{CE}\ (\%) = \frac{\text{n} \times \text{F} \times \text{V} \times \Delta\text{TOC}_\text{E}}{12000 \times \text{mQ}} \times 100 \tag{4}$$

Where, $\mathrm{TOC_i}$ and $\mathrm{TOC_f}$ are the initial and final total organic carbon content of wastewater in mg L^{-1}. $\Delta\mathrm{TOC_E}$ is the experimental depletion of the total organic carbon content of water in mg L^{-1}; n is the number of electrons (78) required complete mineralization of CIP as per eq (5); F is the Faraday's constant (96487 C Mol^{-1}); V is the effective volume of reactor; Q is the total charge consumed during the process (I×t); 12000 is the molecular mass of carbon in mg L^{-1} and the number of carbon atoms in 1 mole of CIP (m=17).

$$\mathrm{C_{17}H_{18}FN_3O_3 + 34H_2O \rightarrow 17CO_2 + 78H^+ + NO_3^- + 2NH_4^+ + F^- + 78e^-} \qquad (5)$$

2.4. Evaluation of residual toxicity

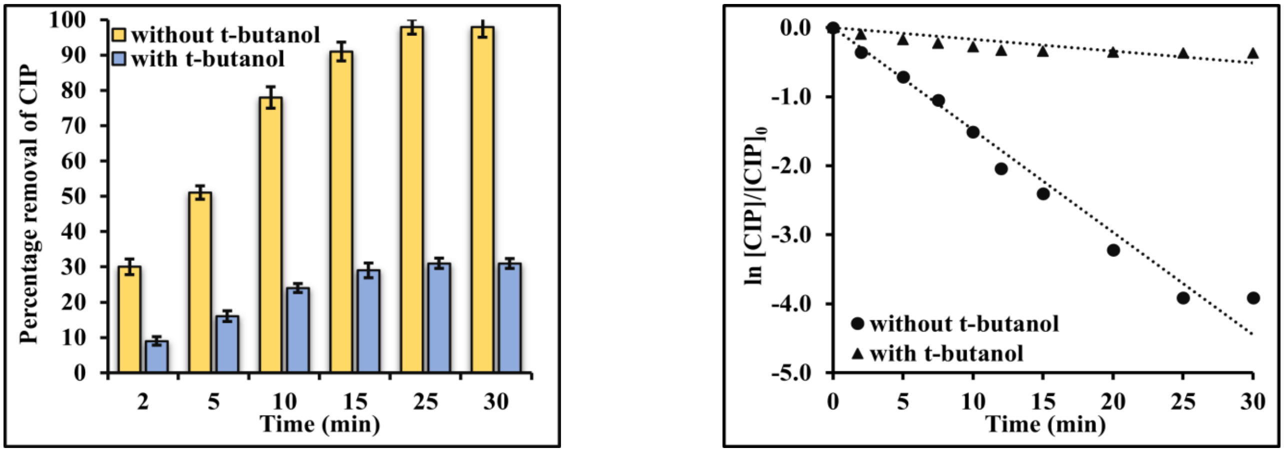
The investigation into the toxicity variations of 1 mg L^{-1} raw CIP and EAOP-treated effluent (50% degraded and final effluent) involved assessing the growth of *E. coli* through the optical density method at a wavelength of 600 nm. Using MUG agar, *E. coli* was isolated from municipal wastewater collected from the drain located near the First Gate of IIEST, Shibpur, Howrah. Subsequently, a stock culture of *E. coli* was prepared in lactose broth (LB). For the study, 10 test tubes were prepared, each containing 5 mL of LB and 100 µL of the bacterial culture. Raw and EAOP-treated CIP were diluted in the ratio of 16:1 and 32:1 (sterilized water: sample) and introduced into these tubes to the same v/v ratio as LB. Additionally, two control tubes were created: one containing only sterilized water with LB and culture and the other containing sterilized water with LB but without culture. The test tubes were incubated for 24 hours at 37 °C, after which the growth of *E. coli* was evaluated by measuring the optical density of the culture at 600 nm (OD_{600}).

3. Results and discussion

3.1. Degradation of CIP by EAOP

The degradation of CIP by EAOP was studied in a batch reactor having a total volume of 250 mL The spacing between C/PbO_2 and steel electrodes was 2 cm. The concentration of CIP was 1 mg L^{-1}. A rapid degradation of CIP was observed initially and then it slowed down (Fig. 3a). About 50 % of the CIP was depleted within 5 min and 98% degradation was achieved in 30 min. The electrogenerated $OH^\bullet$ attacks the CIP molecule vigorously, and hydroxylation occurs in the quinolone moiety, leading to the generation of reaction intermediates [29]. In the later stages, the degradation rate tends to decrease due to the side reactions of $OH^\bullet$ with the intermediates alongside CIP. The degradation followed a first-order reaction model and the rate constant was determined to be 0.148 min^{-1} (Fig. 3b).

To examine the mechanism of CIP degradation, $OH^\bullet$ quenching study was done using t-butanol (tert-butyl alcohol). Upon adding t-butanol, the degradation was reduced to 31% which was observed to be 98% without t-butanol (Fig, 3a). This indicated that the major degradation of CIP was due to $OH^\bullet$. While the rest was associated with the direct anodic oxidation pathway. The reaction rate was decreased from 0.148 to 0.018 min^{-1} (Fig. 3b). Similar results can be seen in the study by Cai et al. (2019) where the addition of t-butanol reduced the degradation efficiency during the EAOP of di-n-butyl phthalate (DnBP) to 21% from 91% using BDD anode [30].



(a)

(b)

Fig. 3(a): Degradation of CIP during EAOP and **(b)** linearized plot of pseudo-first-order kinetic model [initial conc. 1 mg L^{-1}; pH 3(±0.5); current density 30 mA cm^{-2}; spacing of electrodes 2 cm; 150 mM Na_2SO_4 as electrolyte]

3.2. Mineralization of CIP and residual toxicity of treated effluent

It was observed that the EAOP could reduce the CIP to a significant level. However, it is necessary to examine the mineralization efficiency and the toxicity of the effluent. Therefore, the TOC of the treated samples was determined. The TOC removal efficiency was observed to be increased during the process and attained a maximum of 85% at 30 min (Fig. 4). This elucidates the fact that the organic carbon present in the matrix was being converted into inorganic form, mainly CO_2 and H_2O, which are the simplest end products of any organics, as per eq. (6). More mineralization indicates a smaller amount of partially oxidized byproducts, which leads to a reduction in the residual toxicity of effluent. The mineralization efficiency obtained using EAOP was observed to be higher than that of other AOPs like UV/H_2O_2, and UV/H_2O_2/Fe^{2+} which were 29% and 55% respectively [31].

$$\mathrm{Organics} + \mathrm{OH}^{\bullet} \rightarrow \mathrm{CO_2} + \mathrm{H_2O} \qquad (6)$$

The current efficiency, as indicated by eq. 4, was determined to be 42%. This suggests that the current applied is subsequently utilized by side reactions that occur during the generation of byproducts, in addition to the mineralization of the CIP. The more the applied current less will be the efficiency as the extra current will be utilized for the side reactions and oxygen evolution which was seen in the study of Wang et al. (2016) [32].

The toxicity of the residual byproducts and reaction intermediates was assessed, as mentioned in section 2.4. The OD_{600} values for the 50 % degraded samples were found to be lower than the raw CIP solutions (Fig. 5). In contrast, for completely degraded samples, the absorbance was increased, indicating a higher growth of *E. coli* and lower toxicity. During the initial stages of the EAOP process, the generation of reaction intermediates was responsible for the enhanced toxicity. After sufficient mineralization of CIP, the toxicity-causing intermediates were removed. This again reduced the overall toxicity of the effluent. It is reported that different byproducts like phenol amine derivatives are produced during the AOP degradation of fluoroquinolone antibiotics [33]. These byproducts which are formed through defluorination, decarboxylation, or hydroxyl addition to the quinolone ring are mostly responsible for increasing the toxicity of effluent at the initial stages of the treatment. At later stages, the production of less toxic carboxylic acids increased the growth of *E. Coli*, i.e., reducing the toxicity [19]. Therefore, it can be stated that EAOP treatment will help to reduce the toxicity of CIP in wastewater and result in lowering the environmental risk.

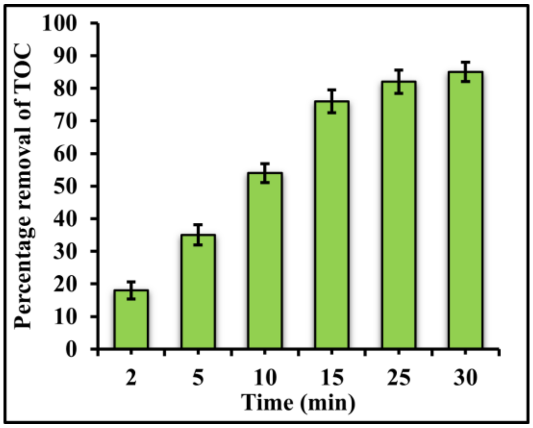


Fig. 4: TOC removal during degradation of CIP by EAOP [initial conc. 1 mg L^{-1}; pH 3(±0.5); current density 30 mA cm^{-2}; spacing of electrodes 2 cm; 150 mM Na_2SO_4 as electrolyte]

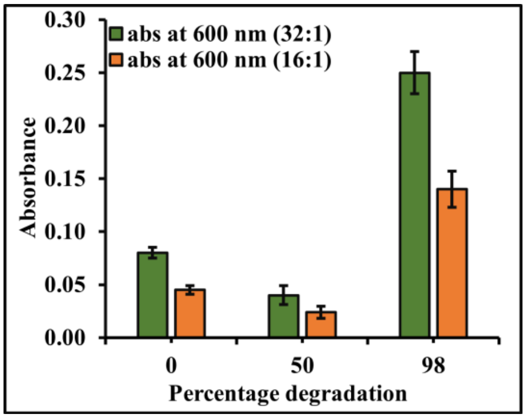


Fig. 5: Changes in absorbance at 600 nm during different stages (50% and 98%) of EAOP [initial conc. 1 mg L^{-1}; pH 3(±0.5); current density 30 mA cm^{-2}; spacing of electrodes 2 cm; 150 mM Na_2SO_4 as electrolyte]

4. Conclusion

The EAOP, using C/PbO_2, as an anode successfully degraded the CIP from wastewater. The degradation was very fast at the initial stages showing 50% degradation at 5 min when initial CIP concentration was 1 mg L^{-1} at a current density of 30 mA cm^{-2}, with an electrode spacing of 2 cm and pH of 3±0.5. Almost 98% degradation of CIP was achieved within 30 min. The degradation kinetics followed a pseudo-first-order model, and the rate constant was determined to be 0.148 min^{-1}. Alongwith CIP degradation, the TOC removal was found to be 85%, indicating effective mineralization of the reaction intermediates. The current efficiency of the EAOP process was calculated to be 42% which indicated the usage of applied current in both the mineralization and the formation of intermediates. Furthermore, the final toxicity of the treated solution against *E. coli* was found to be lower than that of the influent matrix, suggesting the successful removal of toxic byproducts and a decrease in the ecological risks associated with the specific pollutant. Therefore, the EAOP process can be applied for the treatment of pharmaceutical wastewater.

5. Acknowledgments

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