DEGRADATION OF CIPROFLOXACIN USING FENTON PROCESS AND ITS EFFECT ON BIODEGRADABILITY

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ABSTRACT

Ciprofloxacin (CIP) is a second-generation fluoroquinolone antibiotic used for the prevention and treatment of infections in humans as well as in animals. As an emerging contaminant in the environment, its main source is the pharmaceutical industry and municipal wastewater treatment plants. The contaminant surpasses the conventional treatment process and end up in surface water bodies and cause toxic to aquatic organisms and generate antibiotic resistance. Hence, the degradation of CIP and its effect on biodegradability using an advanced oxidation process such as the Fenton process was investigated in the present study. A maximum of 71% removal of CIP was achieved from an aqueous solution of CIP with 10 mg/L of initial concentration at 60 min with an optimum concentration of Fe (II)/H₂O₂ ratio of 1:10 at pH = 3. Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) were determined to find the biodegradability of the aqueous solution during the treatment process. The process enhanced the biodegradability of the solution with a considerable reduction in COD and an increase in BOD values.

Keywords: Ciprofloxacin, Fenton Process, Biodegradability, Degradation

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INTRODUCTION

Pharmaceutical industries are outspreading their roots from the mid-1800s through inventions and applied research work. Pharmaceutical compounds consist of a vast community of human and veterinary medicinal compounds that are being used globally for a long time.^{1,2} These compounds are designed to interfere with human and animal hormone systems as a cure to human health at nano concentration levels and therefore as a key property, they are designed to persistent against biological degradation. Pharmaceuticals enter the environment in several ways (i) from the excretion of humans and animals via urine and fecal products (ii) from industrial effluents, disposals from hospitals, and agricultural runoff. In sewage treatment plants, pharmaceuticals are still not eliminated and are instead discharged into receiving water systems. Thus the prevalence of these pollutants in surface water and subsurface waters that are organic and inorganic by nature have resulted from sewage treatment plants, polluted soils, farm runoff, industrial wastewaters, and storage leakage. Many organic compounds are known as harmful and hazardous even if they are detected at very low doses.³ With the development in measuring technologies, the pharmaceuticals are being detected and identified even in trace level concentrations. The quantity of these drugs in the environment is minimal, but their continued persistence in the environment can pose a significant long-term risk. Therefore, in recent years these are considered an emerging environmental problem.⁴

Antibiotics are a group of pharmaceutical compounds used as human and veterinary medicines to treat bacterial infections. Out of these, ciprofloxacin (CIP) is a commonly used antibiotic and its production and use are very high in a country like India.⁵ After production and use, the effluents from industries and sewage treatment plants have to be treated properly to remove this contaminant.⁶ The compound by its origin, self-retain their chemical structure and are non-biodegradable, so its existence in the environment for a longer period is considered to be a threat for life.⁵ The existence of such chemically resistant compounds in water creates a major threat to public health because they are poisonous, damage endocrine system, mutagenic, or possibly carcinogenic³. While the concentration of such pharmaceutical compounds is very less in the aquatic environment, their constant presence may create a possible long-term danger to aquatic and terrestrial species.¹

The complex structure of CIP cannot be removed by the biological treatment process and therefore require an advanced oxidation process (AOP) for its degradation. These special treatment methods employ chemical processes involving Fenton's based oxidation, UV irradiation, photolysis, photochemical treatment, electrochemical treatment, sonolysis, photocatalysis, etc. In these processes, hydroxyl radicals are generated via the removal reactions.⁷ AOPs can be used either individually or in collaboration with certain traditional procedures of treatment, based on the characteristics of the wastewater to be treated and the quality of the effluent required. For example, AOPs can be carried out as a pre-treatment step to transform bio recalcitrant compounds to intermediates that are more readily biodegradable, accompanied by conventional methods of treatment.¹

In this present study, advanced oxidation using Fenton's reagent was used for the degradation of CIP. This method involves Fenton's reagent which constitutes ferrous ions and hydrogen peroxide and Fe^{2+} act as a catalyst to degrade or treat higher molecular structures to simple organic compounds. Formation of hydroxyl radicals and ionization of ferrous ion from Fe^{2+} state to Fe^{3+} act as coagulants and thereby both oxidation and coagulation occur in this method of treatment. Fenton reaction occurs according to the equation-

There is proof that the reaction involved in the Fenton reaction is much more complex than that described in the equation⁷. The reaction is expected to complete in a short time for the Fenton procedure, depending on the concentration of H_2O_2 and Fe^{2+} . The absolute destruction of COD occurs in a time interval of 5 minutes in comparison to the initial COD. Hydroxyl radicals typically target all compounds, including hydrogen peroxide, in wastewater.⁷ The production and operating costs for implementation of the reactor using Fenton's reagent are considered to be lower relative to the other AOP's.⁴ Degradation of CIP with Fenton's reagent can be easily adopted in treatment plants since both the reagents are cheap, ecofriendly, and easily available. So, the present study aims to find (i) the degradation rate of CIP from an aqueous solution using Fenton's reagent (ii) Factors affecting degradation (iii) the rate of change of biodegradability of CIP during Fenton's process.

EXPERIMENTAL

Material and Methods

The analytical grade Ciprofloxacin (99.9% purity) was acquired from Sigma-Aldrich, and 10 ppm solution was made by using a buffer solution. The buffer solution was prepared with 4% Sodium Hydroxide and analytical grade methanol. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Ferrous sulfate heptahydrate) and Hydrogen peroxide (30% H_2O_2) were purchased from SIS, Chennai. Sodium Hydroxide (NaOH) and Sulphuric acid (H_2SO_4) were used to adjust pH. Syringe filters of 0.45 μm size were used to remove the impurities from the sample before spectrophotometer analysis. The analysis was done in UV-VIS spectrophotometer, (Shimadzu UV 3600 Plus) at Nano Technology Research Center, SRM Institute of Science and Technology, Chennai.

Experimental Procedure for Fenton reactions

The absorption spectrum of CIP was plotted within the wavelength range of 100 to 700 nm. The maximum absorbance was obtained at 262 nm. The calibration curve was plotted with absorbance and different concentrations of CIP (2ppm, 4ppm, 6ppm, 8ppm, and 10ppm). This curve was used to find the concentration of CIP after each experiment.

For further experiments, the initial concentration of CIP was taken as 10 ppm. All the experiments were conducted in a 500 ml glass beaker confined to a reactor volume of 200 ml on a magnetic stirrer as an external energy source for thorough mixing with a running speed of 250-300 rpm. The initial experiments were conducted to find the optimum ratio of Fe^{2+} to H_2O_2 and optimum time for maximum degradation of CIP. The ratio of Fe^{2+} to H_2O_2 was varied from 1:5 to 1:20. The pH was adjusted to 3 and experiments were conducted at room temperature. A sample volume of 5 ml was extracted at different time intervals and was passed through a 0.45 μm syringe filter for analysis in the UV-VIS spectrophotometer. Further experiments were done to find the effect of pH and initial Fe^{2+} concentration.

Experimental Procedure for Biodegradability Test

COD and BOD were determined in the laboratory by using APHA method numbers 5220D and 5210D respectively. The COD was determined by the closed reflux colorimetric test. The required samples were pipetted into the reagent solution and it was refluxed in the COD digester for 2 hours at 150°C. COD was measured by using the Lovibond Spectrodirect instrument calorimetrically. BOD₅ was measured using BD 600 Lovibond BOD analyzer. The required amount of two reagents-nitrification inhibitor and hydrogen peroxide solutions were added to the sample in BOD bottles and incubated for 5 days. The BOD was automatically read from the analyzer after 5 days.

RESULTS AND DISCUSSION

Degradation of CIP using Fenton's reagents was studied for various ratios of Fe²⁺/H₂O₂. The study was conducted to find the effect of various factors on the oxidation process. To find out the effect of Fenton oxidation on biodegradability, BOD, and COD of the solution were tested at different stages. The results of each experiment are discussed in further sections.

Optimum Ratio of Fe²⁺ and H₂O₂ For Degradation of Ciprofloxacin

Fenton's reagent is a combination of Fe²⁺ and H₂O₂. The rate of reaction varies with the concentration of these two reagents. So, it is very important to find out the combination of these two reagents which give the maximum degradation of CIP. The experiment was conducted at different ratios of Fe²⁺/H₂O₂ and the degradation of CIP was found out at various reaction times. The initial concentration of CIP was 10 ppm and the pH of the solution was adjusted with 3 molar H₂SO₄ and NaOH and maintained at 3. In a reactor volume of 200 ml of CIP, the required quantity of Fe²⁺ was added and mixed thoroughly on a magnetic stirrer. The reaction time starts when H₂O₂ was added to the solution. From a continuously mixed reactor, 5 ml of the sample was extracted at different time intervals such as 5, 15, 30, 45, 60, 90, and 120 minutes. Immediately after extraction, the sample was passed through a 0.45 µm syringe filter to remove all the impurities. The experiment was repeated for different ratios of Fe²⁺/H₂O₂ such as 1:5, 1:10, 1:15, and 1:20. The results of the experiments are shown in Fig.-1. It was observed that 30% of degradation happened in the first 5 minutes of reaction time. After that, the reaction was slow until it reaches an equilibrium state. This is because the production of OH[•] radical is faster at the initial stage.⁸ Degradation of CIP increased with an increase in Fe²⁺/H₂O₂ ratio and a further increase in ratio reduced the degradation. The maximum degradation was obtained at 1:10 ratio with a removal efficiency of 71% and further for higher ratios the degradation of CIP was reduced. The decrease in degradation with a higher ratio can be explained by two reasons. (i) With the increase in the ratio of Fe²⁺/H₂O₂, Fe²⁺ is converted into Fe³⁺ at an excess concentration of H₂O₂ concerning Fe²⁺ concentration⁹. This is explained the equation (2).

(ii) OH[•] radical is scavenged by H⁺ and OH⁻ ions and this is explained by the following equation (3)⁹ and (4).¹⁰

The maximum degradation was observed at 60 minutes for all the ratios and after that degradation was minimum indicating that the equilibrium state was achieved at 60 minutes. The same was reported for all the ratios, the degradation of CIP was 35%, 52%, and 50% for 1:5, 1:15, and 1:20 ratios respectively. This shows that for low ratios the degradation was less and again for higher ratios the degradation was decreased. So it is evident from this experiment that the optimum ratio is 1:10. The same results were reported in most of the studies conducted for the removal of CIP by using Fenton's reagent as well as for other antibiotic compounds. In similar studies conducted by Fenton's reagents such as Giri et al, Gupta et al, Sun et al, and Yang et al achieved a maximum degradation of CIP as 74.4%, 70%, 77%, and 56.8% respectively. The relationship between time and degradation is also almost the same when compared with other studies. Shah et al reported the maximum degradation at 80 min.

Fig.-1: Relation between Fe²⁺: H₂O₂ Ratio and Removal of Ciprofloxacin

Effect of pH on Degradation

Among the numerous desirable conditions influencing the reaction rate, pH is of great significance because H₂O₂ and Fe²⁺ generate hydrogen radical in acidic conditions.¹⁰ H₂O₂ by definition is strongly oxidative, Fe²⁺ used in the reaction functions as a catalyst, and the pH of the solution assists in the reaction kinetics.¹⁰ From the previous experiment ratio of Fe²⁺/H₂O₂ and time of maximum degradation was optimized as 1:10 and 60 min respectively. These two values were kept constant to find the effect of pH on the reaction rate. The pH was varied from 2 to 4 and the degradation of CIP was found out. The variation of CIP removal percentage with pH is shown in Fig.-2. From the experiment, it is found that the maximum removal occurred at pH= 3.

The initial pH of the aqueous 10 ppm CIP solution is 8. The pH of the solution was reduced by adding 3 M Sulphuric Acid. No major variations in treatment effects were found when assessed with an initial higher pH level greater than 4.5, while pH 3.0 produced marginally better outcomes. This result is compatible with recent works and results indicate that the ideal pH for oxidation by Fenton's reagent does not depend on the composition of the wastewater but depends on pH in the range of 3-5.¹¹ It's very well accepted that the H₂O₂ + Fe²⁺ reaction will produce radical hydrogen in an acidic environment.¹⁰ Ferrous ions are considered to be not stable at pH>4.0, simple to form ferric ions, and tend to create complexes of ferric hydroxides or ferric oxyhydroxides which do not react with hydrogen peroxide due to its low activity.¹² At high pH, hydrogen peroxide becomes unstable and decomposes to H₂O and O₂.¹⁰ Shah *et al*¹³ also reported that the maximum degradation of CIP was achieved at pH = 2.5 in 80 min. The formation of ferrous and ferric hydroxide complexes at pH 6.0 which had a much lower catalytic capacity than Fe²⁺ and resulted in the generation of a smaller amount of OH radical.¹⁴ This leads to less removal efficiency at higher pH. The optimal pH value for the removal of various antibiotics such as amoxicillin¹⁵, is 3 and ampicillin at pH 3.7.¹⁶

Fig.-2: Relation between pH and Removal of Ciprofloxacin

Effect of The Initial Concentration of Fe^{2+} on Degradation

Among various factors that affect the efficiency of treatment, the concentration of Fe^{2+} is of prime importance. Ferric ions play a vital role as a catalyst in the process, which activates hydrogen peroxide for the production of hydroxyl radicals. An increase in the concentration of ferric ions can cause some degree boost in the development of hydroxyl radicals by hydrogen peroxide.¹⁰ In order to determine the optimum Fe^{2+} , the experiment was conducted by varying concentration of Fe^{2+} such as 10mg/L, 20mg/L, 30mg/L and 40mg/L. The treatment was conducted for a total of 60 minutes handling 200 ml volume in a reactor by keeping all other parameters constant. The relation between Fe^{2+} concentration and degradation of CIP is shown in Fig.-3. The maximum removal was obtained at 30 mg/l of Fe^{2+} concentration and a further increase of Fe^{2+} reduces the degradation. Similar results were reported in various studies also.^{10,17} It means that the excess amount of Fe^{2+} as a scavenger reacts with hydroxyl radicals and form Fe^{3+} which has a negative impact on degradation as in eqn.-2. Kargi et al 2010 also reported the Amoxicillin removal by using Fenton's reagent showed that the maximum degradation was at 25 mg/L of Fe^{2+} , after that there was a reduction in removal efficiency. A study on removal of CIP by electro-Fenton process also reported that an increase in Fe^{2+} concentration till 0.1 mM increased the degradation, further increase reduced the degradation.¹⁸

Fig.-3: Initial Concentration of Fe^{2+} and Its Effect on the Removal of Ciprofloxacin

Effect of Fenton's Process on Biodegradability

Most sewage treatment plants use the activated sludge process as their prime treatment method to remove organic materials from wastewater. But these biological processes do not completely remove pharmaceutical compounds such as CIP. The average removal efficiency of fluoroquinolones was approximately 65% during conventional wastewater treatment.¹⁹ CIP is a chemical compound and it is not degraded by biological processes. Fenton's process improves the biodegradability of the solution, hence CIP can be removed by the biological process from the wastewater. The biodegradability of the solution can be determined from BOD_5/COD ratio. If the BOD_5/COD ratio is greater than 0.4, the solution is biodegradable. Thus the overall efficiency of the treatment can be improved by a combined process such as Fenton's process coupled with a biological process. Fenton's process can be used as a pretreatment process that can be used to improve wastewater biodegradability. Hence the variation of biodegradability of the aqueous solution of CIP was determined during the oxidation process.

To evaluate the biodegradability, the BOD_5/COD ratio was found out at different times of the degradation process. The solution is biodegradable when the BOD_5/COD ratio greater than or equal to 0.4.²⁰ The initial BOD of the solution was zero indicates that the solution is non-biodegradable. The BOD_5 and COD results are shown in Fig.-4. COD of the solution was considerably reduced during the Fenton process and BOD increased till 60 minutes after that there is a reduction in BOD. The maximum degradation of CIP was also achieved at 60 minutes. Gong et al 2015²¹ also reported, similar results in the degradation of levofloxacin using the electro Fenton process. Biodegradable intermediates were formed during the process and it enhances the BOD_5 of the solution. Variation of BOD_5/COD with time is shown in Fig.-5. At the starting of the experiment, BOD_5/COD ratio was 0.04 which shows the non-biodegradability of the solution. The ratio

further increased to 0.4, indicating that the solution becomes biodegradable at 60 minutes and after that, there was a reduction in the ratio.

Fig.-4: BOD₅ and COD Variations during Degradation of Ciprofloxacin using Fenton's Process

A study conducted on enoxacin degradation by the electro-Fenton process also reported that longer reaction time reduces biodegradability.²² It can be explained that the biodegradable intermediates formed in the initial stage of the process are easily removed by the hydroxyl radicals and hence there is a reduction in BOD. It can also be noted that the Fenton process transforms the structure of the pollutant into less toxic and make it biodegradable rather than mineralization. The results showed that 100 percent removal of pharmaceutical contaminants can be achieved when the Fenton process is used as a pretreatment method followed by a cheaper biological process in wastewater treatment plants.

Fig. -5: Variation of BOD₅/COD Ratio with Time

CONCLUSION

This experiment was conducted to address one of the cost-effective ways to remove ciprofloxacin antibiotics which are contaminating the various water sources since treatment plants are not effective in removing ciprofloxacin antibiotic specifically. From the above work, it can be concluded that:

- The operating parameters which affect the degradation of CIP are pH, Fe²⁺ concentration, H₂O₂ concentration, and the ratio of Fe²⁺/H₂O₂.
- The optimum ratio of Fe²⁺/H₂O₂ for the maximum degradation of CIP was 1:10. The maximum degradation was also achieved at 60 minutes of reaction time at a pH value of 3.
- 71% percentage of degradation of ciprofloxacin was achieved under optimum conditions.
- There was a considerable reduction in COD and an increase in BOD values which shows that the biodegradability of the solution was increased during the process. The solution becomes biodegradable and suitable for the biological process. This treatment process is suitable for wastewater with high COD content.

- Fenton process can be used as a pretreatment method in treating industrial wastewater coupled with the biological process so that the organic contaminant level in the effluent can be effectively reduced thus natural resources can be protected from pollution with emerging contaminants.

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