Research Article

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Oxidative degradation of gentamicin present in water by an electro-Fenton process and biodegradability improvement

Abstract: In the context of environmental protection, where there is a need to develop effective operations for carrying out appropriate treatment of polluted water by pharmaceuticals. Therefore, the present study aims at evaluating the degradation for gentamicin through electro-Fenton (EF) operation, through taking into consideration the effect of several parameters of experimental in the process, namely, the concentration of initial gentamicin, the applied current and the Fe^{2+} (II) quantities. The (EF) operation employed involves a carbon-felt as cathode and platinum as anode at pH 3. Studies for the gentamicin kinetics is monitored by HPLC giving a pseudo-first order reaction following by a chemical oxygen demand, with a reached degree of mineralization 96% after of four hours of treatment through current 100 mA/cm² with 0.1 mM of Fe^{2+}. We find that the degradation for molecule of gentamicin is accompanied by an augmentation of the biodegradability, assesse through the Biochemical Oxygen Demand (BOD5) on chemical oxygen demand (COD) ratio, that augmentation from 0 to 0.41 before treatment after 30 min for EF treatment, showing that there is potential for conjugation of the EF process and the biological process. Furthermore, the by-products have been identified on the basis of HPLC-MS/MS results.

Keywords: Antibiotic; kinetics; electro-Fenton; degradation; biodegradability.

1 Introduction

The existence of emerging contaminants in the effluents is a concern on the environment and human health. For example, antibiotics. The stability and the polarity of these compounds allows them to exist highly moving in the water environment and thus reach drinking water through groundwater leaking. The constant organic pollutants have been found in wastewater effluent, and in surface and drinking water [1].

Gentamicin sulfate is an broad spectrum antibiotic that is active against both Gram-positive and negative bacteria. And Gentamicin C1 has a methyl group in the 6’ position of the 2-amino-hexose ring and is N methylated at the same position [2,3]. Gentamicin is a mixture derived from fermentation containing four main compounds: gentamicin C1, C1a, C2, C2a, and a small compound for gentamicin C2b. Gentamicin and its linked material are highly polarized compounds with high solubility in water and low solubility in most other organic solvents and they all contain a chromophore for ultraviolet light.

Since the end of the last century, fresh water scarcity has become prominent. The global water consumption has increased substantially. The water withdrawals for all the industries account for 32% of the total volume sampled on the water resources [4] and the industrial processes are currently still responsible for half of point source discharges of the organic pollution in the environment.

Therefore, the treatment of water contaminated with organic pollutants is therefore required in order to save water and to protect the quality of our groundwater resources and surface water. In the case of water contaminated by the constant organic pollutants, the utilization of advanced oxidation is becoming more common as a water treatment compared to the conventional (or universal) methods which are overall ineffective.

There are many studies for gentamicin including a degradation study of gentamicin by UV spectroscopy [5].
Biodegradation for gentamicin through bacterial consortia AMQD4 in in the wastewater and raw gentamicin [6].

In this regard, the effectiveness of the advanced electrochemical oxidation process “Electro- Fenton” for the degradation of the toxic organic and/or the persistent pollutants in aquatic media was well demonstrated in the literature [7].

This study shows the performance of the EF method for effective removal of the gentamicin in water using an electrochemical cell with a platinum as anode and a carbon felt as cathode.

As explained above, also based the EF operation on the production for radicals of hydroxyl radicals which possess higher oxidizing power than the conventional oxidants (reaction (1)) in acidic media. Also, the reactions (2) and (3) show the Fenton reagents used in the reaction mechanism:

\[
\begin{align*}
H_2O_2 + Fe^{2+} + H^+ &\rightarrow \bullet OH + Fe^{3+} + H_2O \\
O_2 + 2 H^+ + 2 e^- &\rightarrow H_2O_2 \\
Fe^{3+} + 1 e^- &\rightarrow Fe^{2+}
\end{align*}
\]

The reproduction for Fe\(^{3+}\) of electrochemical generation of Fe\(^{2+}\) ions generated through the react (1) and regeneration of \(H_2O_2\) through (the reaction 2) which catalyze continuous production for \(\bullet OH\).

The oxidizing power of the hydroxyl radicals in acidic medium is very high (\(E^\circ (\bullet OH / H_2O) = 2.8 \; V/SHE\), and its reactivity are optimum. The hydroxyl radicals become capable, due to these properties, of attacking any organic molecule present in the solution. Therefore as a final result, we get the complete mineralization in \(CO_2\) and \(H_2O\).

In addition, the idea of coupling the EF process with the biological one appears very interesting from the point of view of the economy and the efficiency [7-11] Indeed, this study shows the possibility for the combining the EF operation and the biological process to eliminate the gentamicin from water. The experimental electrolysis tests were performed on the aqueous solutions of the gentamicin at constant current density with the presence of the Fe\(^{2+}\) as catalyst and \(pH = 3\). As a result, the effects of the current and the concentration for Fe\(^{3+}\) (catalyst) on the kinetics for the degradation and the mineralization of the gentamicin are examined. The degradation was examined by changes in the concentration of the gentamicin and the mineralization of the treated solutions was investigated through the electrolytic were, the chromatographic analysis using high where the chromatographic analyses utilize high performance liquid chromatography (HPLC) and also the mass spectrometry (LC-MS / MS). The BOD\(_5\) / COD values were collected at various times through the EF operation to approximate the time optimum to transition to the operation of biological.

2 Experiential section

2.1 Chemicals required:

The gentamicin (\(C_{21}H_{43}N_5O_7\)) from AFRIC-PHAR licensed obtained. The solutions of aqueous were prepared by ultra-pure water obtained from a Millipore system.

Acetonitrile (analytical grade) from Carlo ERPA was obtained. The rest of the chemicals including sodium sulfate, \(H_2SO_4\), \(FeSO_4\) and potassium chloride from Shangai C. R. Co. (Shangai, China) were obtained.

2.2 The electrolytic method

Voltalab Potentiostat/Galvanostat instrument, type PGZ 301 was employed for the electrolysis, comprising a cell of open with a capacity of 250 mL. A surface of platinum (2.5 cm × 2 cm) was used as anode and three-dimensional surface of (6 cm x 5 cm x 0.5 cm) carbon felt (cathode). The solution consisted of concentrations of gentamicin 0.1 mmol / L, of \(Fe^{2+}\) 0.5 mmol / L and of \(Na_2SO_4\) 0.05 mol / L. The electrolyte (200 ml in distilled water) was maintained at pH 3, and was subjected to a constant agitation. The current density was in the range of 60 to 300 mA / cm\(^2\) at room temperature.

2.3 Analytic procedures

The chemical oxygen demand (COD) is an approach that aims to examine the degradation of the gentamicin in water. The evaluation of the COD was performed using the spectrophotometer (DR . 125) from (Hach Company USA). The measurements read were made using the using the method of dichromate. The medium of reaction was incubated about 120 min at 150 ° C in the Lovibond® COD VARIO photometer.

The evolution of the gentamicin concentration was follow up by invert HPLC used a Waters 2695 coupling photodiode detector, selecting the an optimal wavelength.
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... of 270 nm and adjusted with a C 18 thermosupersilon, 25 cm,5 μm, 4.6 mm (id), column was about 40°C. The analysis on a 100 μl sample volume was carried out isocratically used a mixture of pH=2.2, with mobile phase (acetonitrile 46:54 (v / v)) at a flow rate of 1.5 ml/ min.

The identified of the degradation by products resulting was carried out through LC-MS / MS (AB Sciex-API 3200 QTRAP®, triplex quadrupole instrument) operating by negative ion mode. Have been getting data thorough by the analysis software ©Version 1.5. response aliquots were infused direct in the ion source at a average of 20 μl / min by utilize a micro-syringe (Hamilton corporation, Reno, USA). The model ESI situation are: heated capillary temperature 300°C; sheath gas (N₂) at a flowing average of 20 ml / min; voltage spray -4.5 kV; The gas average and the nebulization gas curtain of the apparatus were adjusted to 30 and 10 ml / min, respectively.

We used an OxiDirect to measure the BOD values with respirometric evaluation method in 5 days (BOD₅). This procedure is performed in dark conditions (20°C, 6.5<pH<7.5). The nitrification inhibitor was N-allylthiourea (Rodier, Legube and Merlet 2009). KOH grains was added to the to bottles in order to trap CO₂. Activated sludge was obtained through a sewage treatment plant (Oneep: office national d’électricité et d’eau potable, Rabat, Morocco).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussions

3.1 Influence of the applied current in aqueous solutions on the oxidative-degradation of the gentamicin

There are several parameters influencing the EF process; among them are: the intensity of applied current, the concentration for Fe²⁺, and the pH of the electrolyte. It is noteworthy that the best conditions include optimum pH about 3 [12-14] and sodium sulfate as support electrolyte [15]. The study of the applied current effect as examined for 200 ml of the gentamicin concentration of solution 0.1 mM with 0.1 mM for Fe²⁺at about (pH 3), Through (EF) operation is showing into Figure 1.

The increase for the applied current from 60 until 100 mA/cm² leads toward an acceleration of the degradation kinetics of the gentamicin, which can be explained by the acceleration of the rate of electrochemical reactions (2) and (3) leading to radicals •OH (reaction of Fenton (1) in pH= 3). Figure 1 explains that the kinetic oxidation curves of the gentamicin during EF treatment follow an exponential
decrease in concentration during the electrolysis. The treatment time for the complete disappearance of the gentamicin is 60, 13 and 20 min for the applied currents 60, 100 and 300 mA/cm² respectively (Figure 1).

Where it is noted that the degradation becomes faster with the augmentation into the applied current, except in the case of 300 mA/cm². It can be said that 100 mA/cm² is the optimal value for the current in the operating conditions (Figure 1).

Confirmation of this result is shown in figure 1, relying on kapp values. Thus, values of kapp for the oxidative of gentamicin through hydroxyl radicals was found simulating pseudo-first order reaction kinetics. The exponential decrease in the gentamicin concentration and the straight streak lines for kapp (find in Figure 1 (a)) are consistent with first-order reaction kinetics of other studies in the field [16]. The elevation about 0.045 to 0.314 min⁻¹ for values of kapp is in synergy in addition to the improvement of the produced rate of radicals ‘OH electrochemically. The kapp value associated with 300 mA/cm² is decrease than of 100 mA/cm², confirming reason for selection of 100 mA/cm² where they are considered the best values in order to degrade gentamicin.

Indeed, by applying a higher applied current we might accelerating the reactions rates as the reduction of O₂ to water (reaction (4)) instead of H₂O₂, the increase of H₂ (reaction (5)) and the oxidation of H₂O₂ (reaction (6)) [17].

\[
\begin{align*}
O_2 + 4 e^- + 4 H^+ & \rightarrow 2 H_2O \quad (4) \\
2 H_2O + 2 e^- & \rightarrow H_2 + 2 OH^- \quad (5) \\
H_2O_2 & \rightarrow O_2 + 2H^+ + 2 e^- \quad (6)
\end{align*}
\]

3.2 Influence of the experiential parameters on the mineralization gentamicin in solution

There were several experiments performed, by changing from one test to another the applied current in the presence of Fe²⁺ 0.1 mmol / L, on the aqueous gentamicin mineralization kinetics using the EF process (Figure 2 (a)). A decrease in the mineralization is accentuated by increasing the current intensity of the current from 60 to 100 mA/cm². Beyond 100 mA/cm², an increase in applied current density leads to very slow removal kinetics of the COD of gentamicin by the acceleration of the waste reaction (4) which can damage the production of Fenton reagents. Thus, the current of applied of 100 mA/cm² is an optimum value for a maximum mineralization rate for the gentamicin. The mineralization degree is 86% for a current of 60 mA. This degree increases to 96% for 100 mA/cm². Furthermore, sundry experiments were performance by changing the Fe²⁺ concentrations in the range of 0.1 to 0.5 mmol / L at 100 mA/cm² (Figure 2 (b)). According to this figure, we deduce that the optimal degree of COD is attributed to the concentration of 0.1 mmol/L, because this is the best mineralization degree (96%) among other concentrations. From this figure, it can also be observed that the rate of mineralization is rapid by the presence of Fe²⁺ 0.1 mmol / L catalyst while the increase in the concentration for this latter implies a decrease in the effectiveness of the mineralization. This trend may be explained on the basis of reaction being parasitic between the hydroxyl radicals and Fe²⁺. Indeed, at a high concentration for the catalyst, the percent of reaction of the hydroxyl radicals with Fe²⁺ increases (reaction (8)). We therefore conclude that the catalyst concentration (Fe²⁺) is quite crucial in the EF operation and needs to be optimized carefully.

\[
\text{• } \text{OH} + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{OH}^- \quad (8)
\]

Moreover, the instantaneous current efficiency ICE (can be calculated by COD values using the relation (9) [17]:

\[
\text{ICE} = (\text{COD}_0 - \text{COD}_f) \frac{FV}{8It}
\]

Where, COD₀ refer to the starting COD value, COD₁ refer to the final COD value, I is the applied current (A), F is the Faraday constant (96.487 C mole⁻¹), t is the time of treatment (s) and V is the solution volume (L).

The ICE value is a lot lower at the first oxidation operation when the applied current was low and it doesn’t much change at the current increases for all of the current values. At a higher applied current, higher ICE were observed except for 300 mA/cm². The maximum efficiencies were achieved within the first 120 minutes for all situations, and after this time the ICE decreased slowly, suggesting that the by-products formed through this time are more readily oxidized by the hydroxyl radicals than the intermediates form after this time [18,19]. A stepwise decrease for ICE from after 120 minutes was observed due to the diminution in the oxidation reactions, as can be seen in figure 3. From the figure below, better ICE% values were obtained for 60 and 100 mA/cm², where reach 35% and 45% at 60 min, respectively.
3.2.1 Influence of the gentamicin concentration into the mineralization rate in aqueous solution

The values of COD were determined using different concentrations of the gentamicin under the optimal conditions, as shown in figure 4.

3.3 Identification of intermediate composites

The HPLC and LC-MS / MS analyses allowed us to identify the by-products formed. The chromatograms were obtained in the first 13 minutes of electrochemical treatment. They elucidate that there is an increasing disappearance of the gentamicin and the formation of aromatic intermediates. The concentration of these by-products goes to the maximum, then decreases until the complete disappearance. Table 1 [20,21,22,23] shows the identified by-products, while the figures SI-1 and SI-2 show the HPLC chromatograms and the mass spectra of these intermediates, respectively.

3.4 Biodegradability

As expected, gentamicin before electrolysis was not biodegradable Figure 6), after this, the biodegradation...
reaching 0.2 at 15 min electrolysis for 0.3 mM initial gentamicin concentration at $I = 100 \text{ mA} / \text{cm}^2$ and concentration of $\text{Fe}^{2+}$ (0.1mmol / L) showing that by-products, aromatic intermediate molecules, were still resistant for micro-organisms.

It suggests an increase in electrolysis time to generate biodegradable intermediates. The value 0.41 at 30 min be greater than the reference value of 0.40 for BOD$_5$ / COD ratio, In order for the solution to be so biodegradable the value should be greater than or equal to 0.4 [24]. After 30 min electrolysis, biodegradability increased for a BOD$_5$/COD ratio which indicates the suitability of the previously treated EF solution for biological oxidation and this explains the formation of aliphatic intermediates molecules [16,17,25].

Although, after 180 min of electrolysis time, it became less biodegradable, due to a reduction in the ratio BOD$_5$ / COD from the value 8 to 4, which is in agreement with the literature [26].

### 4 Conclusion

In this study, we used HPLC method to estimate the operation of optimum parameters to the gentamicin oxidation through EF method. The results show that total removal of gentamicin is obtained after 13 min of electrochemical treatment for 0.1 mM of Fe$^{2+}$ and used current of 100 mA / cm$^2$. The oxidation of gentamicin is accompanied by the generation of aromatics and aliphatics.
Table 1: The by-products identified by LC-MS/MS during the mineralization of the gentamicin during 60 min by the electro-Fenton process.

| Intermediate products | Chemical formula | Structure | m/z | Reference |
|-----------------------|------------------|-----------|-----|-----------|
| 1                     | C7H15N2O      | ![Structure](image1) | 142 | [20]      |
| 2                     | C18H36N4O10    | ![Structure](image2) | 468 | [21]      |
| 3                     | C13H28N4O4     | ![Structure](image3) | 305 | [22]      |
| 4                     | C13H27N3O6     | ![Structure](image4) | 322 | [20-23]   |
| 5                     | C12H26N4O4     | ![Structure](image5) | 291 | [22]      |
| 6                     | C19H37N5O7     | ![Structure](image6) | 447 | [21]      |
as intermediate molecules. The increase in treatment time improves biodegradation and higher mineralization, as the BOD$_5$/COD ratio reached 0.41 after 30 min of EF treatment, proving that there is potential for conjugation of the EF operation and the biological operation.

**Conflict of interest:** Authors declare no conflict of interest.

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