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Article

Full Factorial Experimental Design for Carbamazepine Removal Using Electrochemical Process: a Case Study of Scheming the Pathway Degradation

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Carbamazepine is an antiepileptic drug which is considered one of the persistent compounds detected in Malaysian aquatic environment. In this present study, a full factorial experimental design was applied for the analysis of effect NaCl amount, initial concentration of carbamazepine, applied voltage and treatment time on the electrochemical removal percentage of carbamazepine. However, applied voltage was found as the most significant factor with $p = 0.00$ lower than 95% confidence level. The investigation of effect NaCl and Na_2SO_4 on the pathway degradation for carbamazepine was provided using liquid chromatography-time of flight/mass spectrometry (LC-TOF/MS). The results showed that new by-products were identified such chloro-epoxy carbamazepine, and other by-products, were detected in the presence of NaCl and Na_2SO_4 electrolytes. High inhibition percentage of *Escherichia coli* (*E. coli*) bacteria was observed at 24.0 h incubation time for both NaCl and Na_2SO_4 electrolytes after 80 and 20 min of treatment, respectively.

Keywords: electrochemical removal, full factorial experimental design, NaCl and Na_2SO_4 as supporting electrolytes, by-product elucidation, toxicity evaluation

Introduction

The occurrence of some emerging compounds like pharmaceuticals in water samples has been considered as one of the emerging issue in environment chemistry. The widespread presence of pharmaceuticals in the aquatic environment could be attributed to their extensive use in medical practice and incomplete removal in wastewater treatment plants (WWTPs).[1-5] One of these emerging compounds is carbamazepine (CBZ) ($C_{15}H_{12}N_2O$), which is one of the most widely used antiepileptic drugs.

According to many previous studies, carbamazepine is considered one of the most resistant compound for both conventional and advanced wastewater treatment process. Consequently, CBZ was widely detected in influent and effluent of sewage treatment plant (STP), as well as in surface water due to its persistence for degradation.[6-10]

Carbamazepine has been treated by different treatment technologies such as ultrasonication, Fenton's oxidation and ferro-sonication,[11] biodegradation after advanced oxidation process (UV/H_2O_2),[8] addition of aqueous chlorine directly to carbamazepine solution[12] and addition of some oxidizing agent directly into solution such as Mn^{VII} and Fe^{VI}.[13] However, most of these processes have some disadvantages, for instance, UV-light source is harmful to skin and eyes, adding raw materials and/or chlorine solution directly to the solution is also considered risky for the health. Electrochemical oxidation process is widely applied to degrade the organic pollutants as reported in previous studies.[14-17] An important advantage of electrochemical oxidation is the ability to degrade the organic pollutants completely to CO_2 and water.[18]

In electrochemical oxidation process, the electrolyte has

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an important role to generate the oxidizing agent which is responsible for degradation of organic pollutants.

The main oxidizing agents in solution are hydroxyl radicals, OCl^-/HOCl and $S_2O_8^{2-}$ in which the generation of these oxidizing agents depend on the type of electrolyte. The hydroxyl radicals are generated after electrochemical oxidation of water at the surface of anode which is a very powerful oxidant and it seems to be directly involved in the oxidation mechanisms that occur on the surface of electrode.[19-23] Mussa *et al.*[24] have been using NaCl to generate OCl^-/HOCl electrochemically which is strong oxidizing agent used to treat leachate sample and simvastatin. Some other authors used Na_2SO_4 as supporting electrolyte to produce weaker oxidizing agent (peroxodisulfate, $S_2O_8^{2-}$) which is used to treat carbamazepine by electrochemical advanced oxidation process[16] as shown in equations 1-5:

$$2Cl^- \rightarrow Cl_2 + 2e^- \quad (1)$$

$$Cl_2 + H_2O \rightarrow H^+ + Cl^- + HOCl \quad (2)$$

$$HOCl \leftrightarrow H^+ + OCl^- \quad (3)$$

$$OCl^-/HOCl + \text{organic pollutant} \rightarrow CO_2 + H_2O + Cl^- \quad (4)$$

$$2SO_4^{2-} \rightarrow S_2O_8^{2-} + 2e^- \quad (5)$$

Up to now, many studies reported the degradation of carbamazepine or other organic pollutants by varying oxidation ways, but the main question is what are the by-products formed and how to identify these compounds at different electrolytes. However, most of the by-products formed during the photodegradation process are hydroxyl-derivatives of carbamazepine since $OH^{\bullet}$ is the most radical available in the solution after photo oxidation of water. Moreover, some chlorinated by-products of carbamazepine were observed using aqueous chlorine as direct oxidation.[12] In electrochemical oxidation process, the formation of by-products depends on the type of electrolyte in the solution. Brillas *et al.*[19] used boron doped diamond (BDD) electrode with Na_2SO_4 electrolyte for the treatment of diclofenac, however, the main by-products are derivatives of hydroxyl-diclofenac. Mussa *et al.*[14] used graphite-polyvinylchloride (PVC) as anode with NaCl as supporting electrolyte to treat caffeine; they noticed that the main by-products are chlorinated compounds. In the light of this comparative study, the type of electrolyte is so important to determine the pathway of by-product formation during electrochemical oxidation process.

In Malaysia, carbamazepine was detected frequently in water samples; influent and effluent of wastewater, and surface water, this is attributed to incomplete removal of carbamazepine in wastewater treatment. However, the chemical oxidation and biological treatment processes are not effective for this purpose. Therefore, the objective of this present work is to apply an effective and clean treatment process which is electrochemical treatment using graphite as anode for the removal of carbamazepine. The study aims to analyze the influence of the different operating variables on the carbamazepine removal, and to find out the optimum values for this electrochemical treatment process. In addition, this work has also the purpose to analyze and identify the main chlorinated and non-chlorinated by-products in the presence of NaCl and Na_2SO_4 as supporting electrolytes using liquid chromatography-time of flight/mass spectrometry (LC-TOF/MS) instrument. The most effective parameter for CBZ removal was defined by using full factorial design method. Finally, the further gain insights by using NaCl and Na_2SO_4 in electrochemical oxidation process, the generation of by-products and evaluation of its toxicity were also tested after their exposure to the *Escherichia coli* (*E. coli*) bacteria at different incubation time.

Experimental

Reagents

Sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) used as supporting electrolyte were purchased from Merck (Germany) with purities of more than 99.5%. Tetrahydrofuran (THF) (CAS No. 109-99-9), polyvinylchloride (PVC) were purchased from Sigma-Aldrich (USA). Carbamazepine (CBZ) (CAS No. 298-46-4) were obtained from Sigma-Aldrich (USA). The deionized water (DI) used was supplied by EASYPure RODI (USA). HPLC-grade acetonitrile (ACN), HPLC grade acetone, HPLC grade methanol and formic acid (FA) were supplied by Merck (Germany). For toxicity experiments, *Escherichia coli* (*E. coli*) bacteria was obtained from Laboratory of Microbiology, Faculty of Sciences and Technology, University Kebangsaan Malaysia, Bangi, Malaysia and Mueller Hinton broth (nutrient broth) was obtained from Merck (Germany).

Experimental procedures

The reaction was provided using a Pyrex glass vessel (100 mL). The Pyrex glass electrochemical cell (reactor) was placed on a magnetic stirring block to keep its contents well mixed during the experiment. Graphite-PVC pellet and Pt plate were used as anode and cathode, respectively. The distance between the electrodes was approximately 2.5 cm. The electrodes were then connected to a direct current (DC) power supply (CPX200 DUAL, 35 V, 10 A, PSU, AIM-TTI, UK).

The experimental design and setup of the graphite-PVC composite electrode was presented (see Figure S1, Supplementary Information (SI) section).

The Pt metal foil electrode was prepared by using a Pt metal foil (99.98% purity from Aldrich Chemical Co., USA). A 0.5 mm-thick Pt foil was cut into approximately 1 × 1 cm piece. The Pt foil was then connected to a silver wire with silver conducting paint and then sealed to a glass rod. Subsequently, epoxy gum was applied to cover the silver wire connecting surface.

The graphite-PVC electrode was prepared by mixing a weighed portion of graphite powder (100 mesh in size and 99.9% purity from Aldrich Chemical Co., USA) and PVC in 4 mL tetrahydrofuran solvent. The graphite-PVC electrode was then swirled flatly to homogeneous consistency followed by drying in an oven at 50 °C for 3 h. The mixture was placed in 1 cm diameter stainless steel mold and pressed at 10 ton cm^{-2} by using hydraulic machine. A typical pellet contained approximately 95% of graphite and 5% of PVC. The total weight of the pellet was approximately 1.5 g. The graphite-PVC pellet was connected to a silver wire using silver conducting paint and sealed to a glass rode. Subsequently, epoxy gum was applied to cover the silver wire connecting surface.

Analytical methods

The evaluation of removal percentage of CBZ in the solution after electrochemical oxidation process was performed by high performance liquid chromatography (HPLC), from a Waters Chromatograph (Milford-MA, USA). HPLC is equipped with a PerkinElmer 785 A UV-Vis detector and a chromolith Rp-18e, C18 column (5 µm, 100 × 4.8 mm). The monitoring CBZ after treatment was performed in a gradient mode with 0.1% formic acid (FA) in DI water as mobile phase A and methanol as mobile phase B, at flow rate of 0.9 mL min^{-1} and a column temperature of 22-25 °C. Each run started with an initial mobile-phase composition of 0% B and was then linearly increased to 100% B over 4 min and then kept isocratic for 1 min. The volume of injection was 20 µL in all experiments. Detection of CBZ was made at 283 nm wavelength. The retention time of CBZ was 2.76 min and the total run was 5 min. The treated solutions were withdrawn after 10 and 40 min then filtered using 0.45 µm filter syringe before injection by HPLC, which is used to monitor the removal% of carbamazepine.

For monitoring the by-products of carbamazepine, LC-TOF/MS instrument was used for this purpose. The treated solutions, withdrawn every 20 min for 160 min.

Samples from the degradation experiments were separated on a Dionex Ultimate 3000/LC 09115047 (USA) system. The chromatographic separation was performed on a Thermo Scientific C18 (250 × 2.1 mm, i.d. 5 µm) column. The injection volume was 30 µL. CBZ and its by-products were analyzed in positive ion (PI) mode (negative mode gives very poor fragmentation). Carbamazepine and its by-products were eluted off the column with a mobile phase consisting of (A) 0.1% formic acid in DI water and (B) ACN-MeOH (3:1, v/v) at 0.3 mL min^{-1}. The elution started at 5% B and was then linearly increased to 60% B over 3 min, further increased to 97% B over 3 min, and then kept isocratic for 5 min. Next, the elution was returned to its starting conditions over 11.1 min and allowed to equilibrate for 5 min prior to the next run. The mass spectrometry was carried out on a TOF instrument (Bruker, Germany) equipped with a Z-spray electrospray interface. The results were obtained with the following settings: MS capillary voltages, 4000 (PI); collision energy for diclofenac, 10 eV; drying-gas flow rate, 8.0 L min^{-1}; drying gas temperature, 190 °C; set capillary, 4000 V; set end plate offset −500 V; set collision cell RF, 250 Vpp and nebuliser pressure, 4.0 bar. The TOF results were collected between *m/z* 50-600. All analytes were acquired using an independent reference spray via the LockSpray interference to ensure accuracy and reproducibility; mixture of sodium hydroxide and formic acid was used as the lock mass *m/z* 90.9766-974.8132. The accurate mass was calculated using software Daltonic Analysis incorporated in the instrument.

Toxicity test

In addition to analysis and identification of the by-products, a toxicity test was also performed for CBZ and its by-products. For this purpose, samples were collected after different times of treatment (0, 20, 40, 60, 80, 100, 120, 140 and 160 min). The response of the toxicity is the inhibition percentage (I%) of the bioluminescence of the bacterium *E. coli* (equation 6) which was determined by Hach spectrophotometer instrument (model DR 2400).

The effect of incubation time (i.e., exposure time to the bacteria) on the inhibition of bacteria was investigated at 1.0, 5.0 and 24.0 h.

$$I\% = \left[1 - \frac{Abs_S}{Abs_C}\right] \times 100 \quad (6)$$

where I% is the percentage of inhibition; Abs_s is the absorbance of sample after different electrochemical degradation treatment times; Abs_c is the absorbance of control sample.

Full factorial design of experiments

This experimental design, which examines NaCl amount (g), initial concentration of CBZ (mg L^{-1}), applied voltage (V) and treatment time (min) factors at once, has been used widely instead of conventional experimental design, which investigates every variable by changing one by one. The experiments require several factors where it is necessary to study the interaction effect of factors on the response (R%).[25] 2^n factorial design of experiments needs less number of experiments for several factors; thus, less cost and time are required.[26,27]

Factorial design states that which factor influences the variation of one factor on the other factors.[25] The four factors were varied at two levels (+1, −1) as shown in Table 1, to investigate their effects on CBZ removal.

Table 1. High and low levels for all four parameters

Factor	Low level (−1)	High level (+1)
NaCl amount / g	0.1	0.2
Initial concentration CBZ / (mg L^{-1})	5	20
Applied voltage / V	3	6
Treatment time / min	10	40

CBZ: carbamazepine.

Experiments for full factorial design were conducted in a 50 mL solution of CBZ. Solutions were always kept between 20 and 22 °C, which was the maximum temperature that can be used in the cell without significant water evaporation during prolonged electrolysis.

A magnetic bar (400 rpm) was used to ensure mixing and to the transport of reactions toward/from the electrodes. However, aliquots of the solutions were withdrawn from the reactor and filtered using 0.45 μm filter syringe, then analyzed either using HPLC or LC-TOF/MS as described in the Results and Discussion section.

Results and Discussion

Full factorial design of experiments

The electrochemical degradation of carbamazepine usually depends on some factors such as NaCl amount, initial concentration of CBZ, applied voltage and treatment time. The results of the experimental design were analyzed using MINITAB 17 statistical software[28] to evaluate the statistical parameters and the statistical plots (Normal probability, Pareto of the standardized effects, main effects, and interactions plots). Forty-eight experiments were tested for CBZ electrochemical degradation using 2^4 full factorial design and three replications of each experiment. The matrix was established according to their high and low levels, represented by +1 and −1, respectively. The coded values of variables with the responses (percentage of removal efficiency) were illustrated in Table 2.

The main effects of CBZ removal were identified based on the *p* value with > 95% of confidence level. The codified equation 7 was used to explain the 2^4 factorial designs of CBZ removal by electrochemical degradation:

$$Y = X_0 - X_1A - X_2B - X_3C - X_4D + X_5AB + X_6AC + X_7AD + X_8BC + X_9BD + X_{10}CD - X_{11}ABC - X_{12}ABD + X_{13}ACD - X_{14}BCD + X_{15}ABCD \quad (7)$$

where Y is the predicted response (removal efficiency in percentage), X_0 represents the global mean, X_i is the regression coefficient corresponding to the main factor effects and interactions, A is the NaCl amount (g), B is the initial concentration of CBZ (mg L^{-1}), C is the applied voltage (V), and D is the treatment time (min).

The main and interaction effects, coefficients of the model, standard deviation of each coefficient, regression coefficients, standard errors, and *t* and *p* values were shown in Table 3.

All the main factors (NaCl amount, initial concentration of CBZ, applied voltage and treatment time) and their interactions were significant at a 5% of probability level ($p < 0.05$). When the factor effect is negative, removal efficiency decreases as the factor is changed from low to high levels (as seen from initial concentration CBZ). In contrast, if the effects are positive, removal efficiency increases to high level of the same factor (as seen from NaCl, applied voltage and treatment time). Furthermore, the fit models, submitted square correlation coefficient (R^2) of 0.9999, were in good agreement with the statistical model.

Figure 1a shows the main effects of the four factors (A, B, C and D) on removal efficiency (%) for CBZ. The effect of a factor is the change in response produced by the change in level of factor. This is frequently called a main effect as it refers to the primary factor of interest in the experiment.[29] It was concluded that the larger the vertical line is the larger the change in removal efficiency (%) when it is changing from level −1 to +1. The model equation related to the level of parameters and removal efficiency was obtained by substituting the regression coefficients in equation 8:

$$Y = 24.69 - 263.6A - 3.053B - 7.420C - 1.7113D + 23.013AB + 82.26AC + 1.189AD + 0.9476BC + 0.23025BD + 0.4436CD - 7.278ABC - 1.2022ABD + 0.7601ACD - 0.067378BCD + 0.33427ABCD \quad (8)$$

Table 2. Design matrix for carbamazepine removal

Run	NaCl amount (A) / g	Initial concentration of CBZ (B) / (mg L^{-1})	Applied voltage (C) / V	Treatment time (D) / min	Removal efficiency (Y) / %
1	+1	+1	+1	+1	70.00
2	−1	+1	+1	+1	0.58
3	−1	−1	+1	+1	48.40
4	−1	−1	−1	+1	0.57
5	−1	−1	−1	−1	0.33
6	+1	−1	+1	+1	97.39
7	+1	−1	−1	+1	9.10
8	+1	−1	−1	−1	1.43
9	+1	+1	−1	+1	2.55
10	+1	+1	−1	−1	0.36
11	−1	+1	−1	+1	4.21
12	−1	+1	+1	−1	11.97
13	+1	−1	+1	−1	40.21
14	+1	+1	+1	−1	14.65
15	−1	−1	+1	−1	16.52
16	−1	+1	−1	−1	0.22

Mean of three replications; CBZ: carbamazepine.

Table 3. Statistical parameters for 2^4 design

Term	Effect	Coefficient	Standard error coefficient	*t*	*p*
Constant		19.9308	0.0532	374.40	0.00
A	19.4450	9.7225	0.0532	182.64	0.00
B	–14.1042	–7.0521	0.0532	–132.47	0.00
C	35.0133	17.5067	0.0532	328.87	0.00
D	18.9167	9.4583	0.0532	177.68	0.00
AB	–1.6392	–0.8196	0.0532	–15.40	0.00
AC	17.2133	8.6067	0.0532	161.68	0.00
AD	12.5767	6.2883	0.0532	118.13	0.00
BC	–12.9375	–6.4688	0.0532	–121.52	0.00
BD	–6.2208	–3.1104	0.0532	–58.43	0.00
CD	15.3950	7.6975	0.0532	144.60	0.00
ABC	1.2142	0.6071	0.0532	11.40	0.00
ABD	3.3975	1.6988	0.0532	31.91	0.00
ACD	11.1117	5.5558	0.0532	104.37	0.00
BCD	–5.8175	–2.9088	0.0532	–54.64	0.00
ABCD	5.6408	2.8204	0.0532	52.98	0.00
S	0.368813				
R-Sq	99.99%				
R-Sq (adj)	99.98%				

A: NaCl amount; B: initial concentration of CBZ; C: applied voltage; D: treatment time; S: standard deviation; R-Sq: R^2 (coefficient of determination).

Equation 8 indicated that all variable interactions were significant, thus, it could not be ignored from the model.

Figure 1b showed that the effect of NaCl amount on removal efficiency (%) was more noticeable when the applied voltage and treatment time were high, but at lower applied voltage and treatment time, effect of NaCl amount was not so high. On the contrary, applied voltage effect was high at lower initial concentration CBZ and long treatment time.

Figure 2a shows the normal probability plot of the standardized effects with $p = 0.05$ to evaluate the significance of each factor and its interactions on removal efficiency (%). Normal probability plot could be separated into two regions, right and left. Right region includes positive coefficients (from C to ABC) while left region includes negative coefficients (AB, BCD, BD, BC and B). The factors symbolized as square are considered significant. Relative importance of the individual and

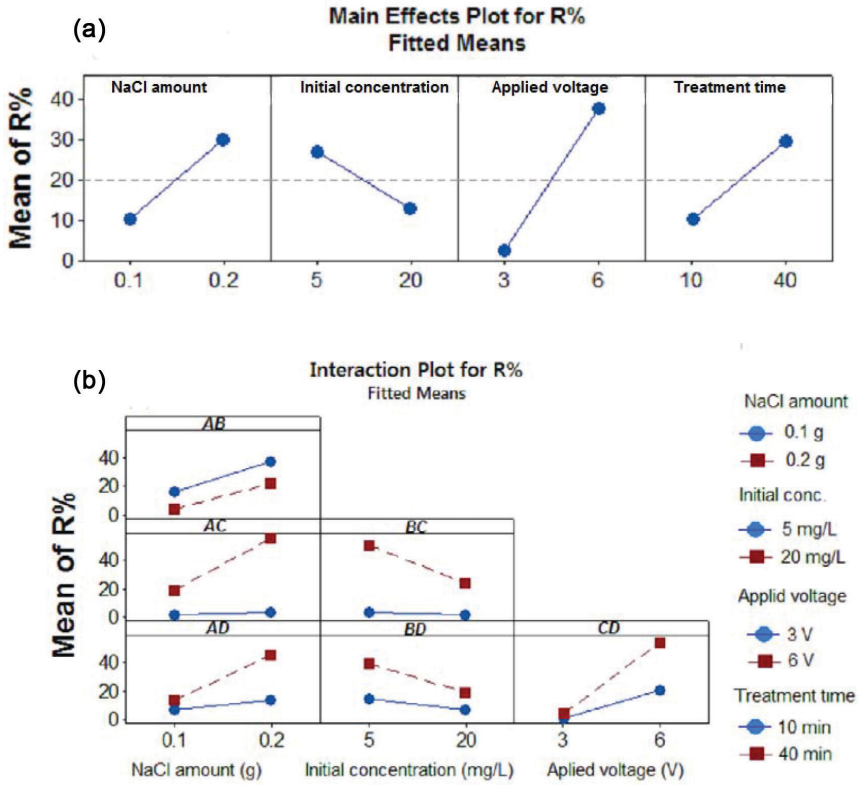


Figure 1. Plots of (a) main effects and (b) interaction effects for the removal efficiency (%) for carbamazepine.

interaction effects was given by the Pareto chart of the standardized effects in Figure 2b. In order to identify whether the calculated effects were significantly different from zero, Student's *t*-test was performed and the horizontal columns in Pareto chart showed the values for each effect. The minimum statistically significant effect magnitude for 95% confidence level is represented by the vertical line in the chart (2.0).

All values higher than 2.0 (p = 0.05), dashed line's right side, are significant. An increase of applied voltage, NaCl amount and treatment time resulted in an uptrend in the percentage removal of CBZ, because the increasing of these factors produces an enough amount of OCl^- as oxidizing agent which participates directly to increase the removal of CBZ. The interaction of two factors AC gives positive indication on removal efficiency of CBZ; thus, an increase in applied voltage and increasing of NaCl amount together resulted in an increase in removal efficiency (%). This antagonistic effect would not be distinguished in the univariate optimization of the CBZ removal process.

Characterization of the by-products

The identification of various emerging compounds and their by-products was reported widely in literature. However, a few studies have been found about the

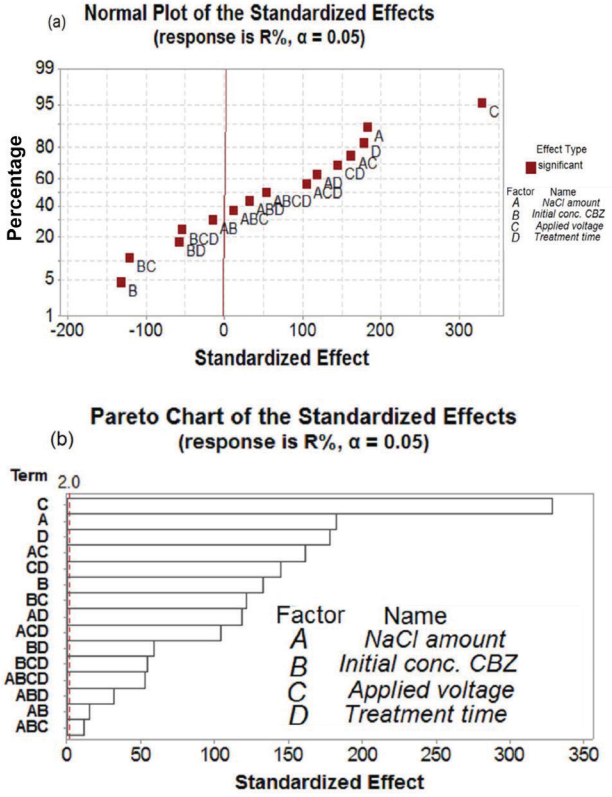


Figure 2. (a) Normal probability plot of the standardized effects at p = 0.05; (b) Pareto chart of standardized effects on the removal efficiency (%) for carbamazepine.

electrochemical oxidation of carbamazepine.[11,16] The analysis and identification of the by-products were achieved using liquid chromatography-time of flight/mass spectrometry. All mass peaks of the by-products were extracted at 0.02 Da narrow window using Bruker DaltonicAnalysis software. LC-TOF/MS chromatograms of CBZ after 20-40 min electrochemical oxidation treatment in the presence of NaCl and Na_2SO_4 were presented in Figures 3a and 3b, respectively. It was observed that six main by-products were formed in the presence of NaCl whereas three by-products were produced in the presence of Na_2SO_4 electrolytes.

The nine by-products were separated and identified according to their retention times (t_R) and *m/z* ratio as follow: product **I** and **VIII** at t_R = 7.72 min, product **II** at t_R = 8.21 min, product **III** at t_R = 8.98 min, product **IV** at t_R = 9.11 min, product **V** at t_R = 10.48 min, product **VI** at t_R = 11.01 min, product **VII** at t_R = 5.67 min and product **IX** at t_R = 9.15 min.

A fully understanding of the formation and mechanism pathway of the by-products are presented in Scheme 1 and Figure 4. However, sodium adduct molecular ion $[M + Na]^+$ is the dominant ion in all compounds. Products **I-IX** were numbered on the basis of its retention times during the separation using liquid chromatography.

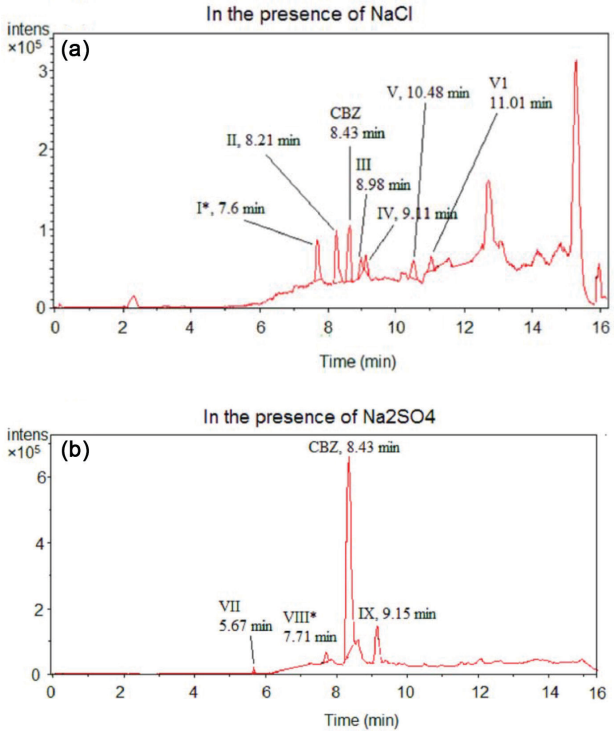


Figure 3. LC-TOF/MS (TIC 100-500 amu) chromatogram of the electrochemical oxidation of carbamazepine: (a) in the presence of NaCl and (b) in the presence of N_2SO_4.

Characterization of by-products in the presence of NaCl

Carbamazepine exhibited a protonated molecular ion at *m/z* 237.0915 (high intensity), and sodium adduct ion at *m/z* 259.0726 (low intensity). Moreover, a minor protonated ion was also observed at *m/z* 194.0872, which resulted from cleavage of the CHNO fragment in the ion source.

Products **I** and **VIII**, as derivatives of hydroxy-CBZ, were formed in the presence of both NaCl and Na_2SO_4, separately at *m/z* 253.0857 and 7.71 min. Three main ions are fragmented from the source ion at *m/z* 236.0586, 210.0816 and 180.0720, which are referring to loss of OH group, CONH and $C_2O_2NH_2$, respectively, as previously suggested by Keen *et al.*[8]

The compound marked as **II** (Figure 4a), whose sodium adduct form presented at *m/z* 309.0248, corresponded to a mass increase of 22 Da with respect to protonated molecular ion of carbamazepine (*m/z* 287.0449). The empirical formula that matches the experimental mass was $C_{15}H_{11}ClN_2O_2$ with an error of 0.33 ppm. From the mass spectrum, product **II** is probably chlorinated CBZ. The most striking aspects of these spectra are the clusters of intense peaks that are each separated by *m/z* 2 Da. However, the isotopic distribution (i.e., presence of an ion at *m/z* 287 (+2 Da) with an abundance of about one-third of that molecular ion) indicated that one chlorine atom is available in this compound. Two more molecular ions are formed at *m/z* 236.0587 and 180.0711, which could refer to loss of NHCl and CO_2N, respectively.

Product **III** is likely to be the product of the cyclation amide group producing a molecular ion with high intensity as sodium adduct (*m/z* 245.0661) and the protonated molecular ion is *m/z* 223.0886. The mass spectra is very clear, which means no more fragmentations, it may be due to the difficulty of the cyclic amide group to give more daughter ions.

A mono-hydroxy carbamazepine is presented in product **IV** showing a sodium adduct molecular ion at *m/z* 277.0700 (+22 Da) (0.19 ppm) and its protonated molecular ion at *m/z* 255.0896. However, fragment ion was detected at *m/z* 209.0849 (–46 Da), which may refer to the loss of H_2N_2O group.

Product **V**, as derivative of diol-CBZ, and product **VI**, as a derivative of chloro-diol-CBZ, have intensity of protonated molecular ions $[M + H]^+$ higher than sodium adduct molecular ions $[M + Na]^+$ as shown from the mass spectra for each one (Figure 4a). However, product **V** gives one mass peak at *m/z* 232.1575 (–40 Da) referring to CNO loss. The mass spectrum of product **VI** indicated a molecular ion at *m/z* 306.1082. The peaks at *m/z* 306 and 308 in Figure 4a, in an approximate of 3:1, indicate that one Cl atom is present in this molecular ion. One additional mass

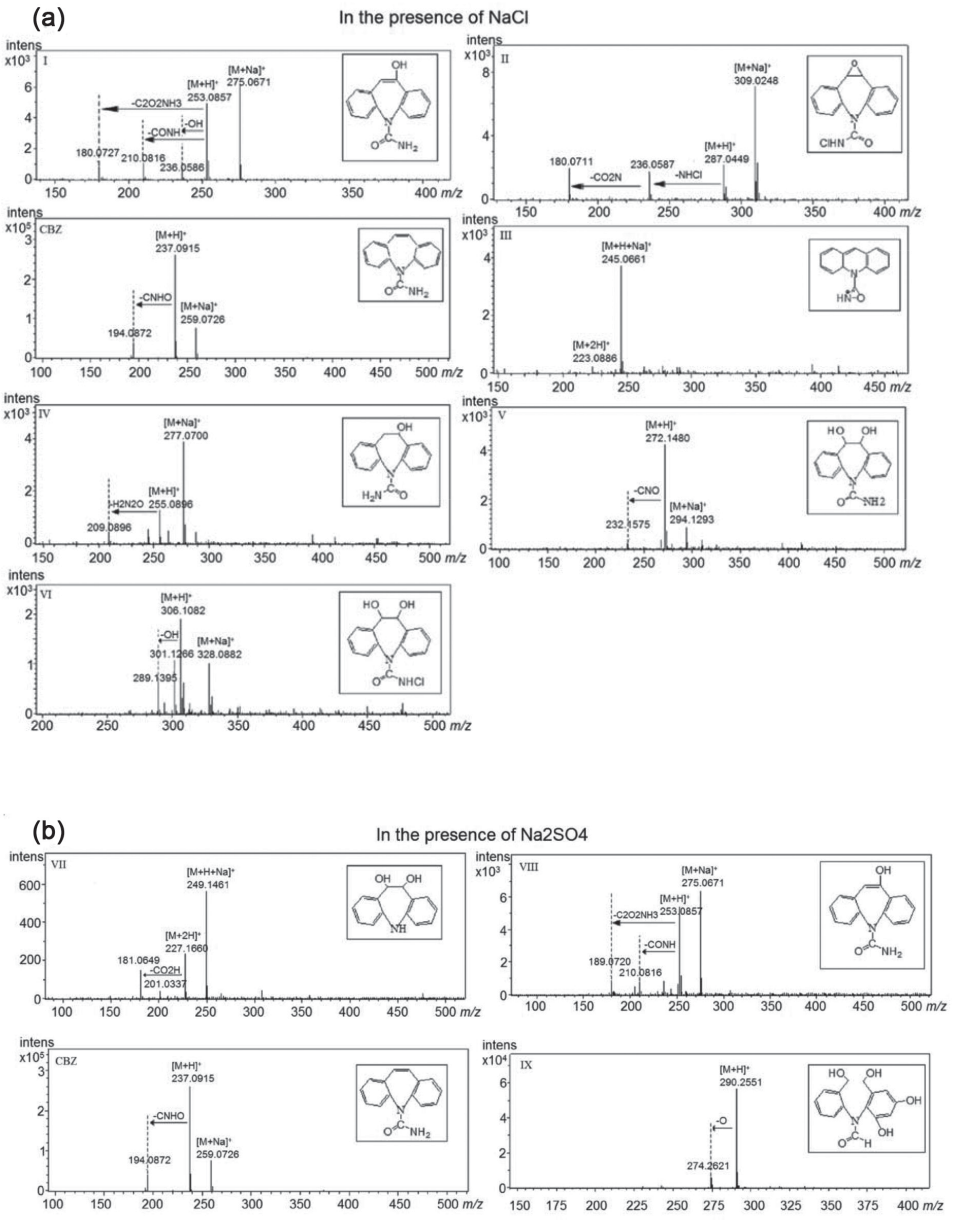


Figure 4. Mass spectra and proposed chemical structures of CBZ and (a) by-products (**I**-**VI**) in the presence of NaCl and (b) by-products (**VIII**-**IX**) in the presence of Na_2SO_4.

peak was present in this product at *m/z* 289.1395 indicating OH loss.

Characterization of by-products via Na_2SO_4

In the presence of Na_2SO_4 as supporting electrolyte, three products were formed (Figure 4b): product **VIII** (discussed before) and products **VII** (derivative of diol-CBZ) and **IX** (derivative of poly hydroxyl-CBZ). The sodium adduct molecular ion is the dominant ion as produced at *m/z* 249.1461 for product **VII**. This product exhibited mass peak at *m/z* 181.0649 which could refer to CO_2H loss. Product **IX** was presented as protonated molecular ion at *m/z* 290.2551 indicating four OH groups substituted on CBZ. One mass peak that appeared at *m/z* 274.2621 may refer to loss of one oxygen atom.

As presented in Scheme 1a, product **I** was identified as hydroxyl-CBZ, which could be produced by attack of hydroxyl radical on the double bond of hepta-ring CBZ producing product **I** ($C_{15}H_{12}N_2O_2$, 0.21 ppm). This product was reported by Keen *et al.*[8] during UV/H_2O_2 advanced oxidation of carbamazepine. Moreover, addition of water molecule to the double bond of the hepta-ring resulted in alcohol-CBZ, which could be converted to epoxy-CBZ (product **II**) after attacking the amide group by chlorine. Mohapatra *et al.*[30] reported that epoxy carbamazepine

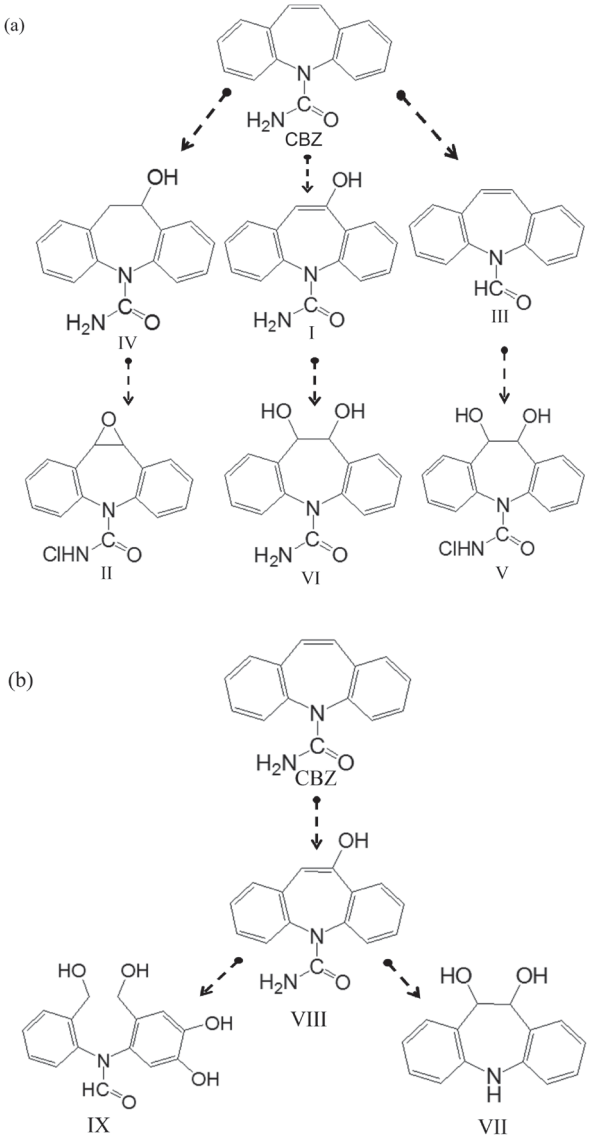
was formed (*m/z* 251.1) during Fenton photo oxidation of carbamazepine, which is matching to the by-product chloro epoxy carbamazepine (*m/z* 287.0449) (product **II**) formed after electrochemical treatment of carbamazepine in the presence of NaCl. However, comparing to the literature, this present study is more reliable in terms of proposed formation of the by-products.

Products **V** and **VI** have been detected as diol-CBZ which have been reported by other previous studies.[29,31] They are produced by the hydrolysis of epoxy-CBZ derivatives. For our knowledge, chloro-CBZ products were formed only in the presence of NaCl meaning that chlorine is released in the NaCl, solution to form OCl^-/HOCl as oxidizing agent. In the presence of Na_2SO_4 (Scheme 1b), there is no more production of chloro-CBZ. Product **VII** (diol-CBZ) is formed after hydrolysis of product **VIII** and losing CH_2NO moiety from its structure. Product **VIII** was determined after treatment of carbamazepine in three different treatment processes: ultrasonication, Fenton's oxidation and ferro-sonication treatment process.[30] Product **IX** is formed by opening the hepta-ring of CBZ-derivative (product **VIII**) to form aliphatic alcohol chain.

In the light of the above results, the type of treatment plays an important role to determine and figure out the produced by-product. However, Andreozzi *et al*.[32] reported that ozonation of carbamazepine produced glyoxal, oxalic acid, oxamic acid, etc., which none of them were formed in this present study.

Toxicity *vs.* by-products

There is a very lack of information in the literature on the ecotoxicity of carbamazepine and its by-products, thus the assessment of the potential risk derived from their presence in the environment is essential. Figure 5 shows the evolution of inhibition percentage (I%) of *E. coli* bacteria as a function of electrolysis time in the presence of NaCl and Na_2SO_4 at different incubation time (1.0, 5.0 and 24.0 h) using a graphite-PVC composite anode. From current results concerning NaCl as supporting electrolyte, the inhibition percentages of *E. coli* bacteria increase to the maximum values of 90, 70 and 40% within 40-80 min at 24.0, 5.0 and 1.0 h, respectively. The inhibition percentage is maximum at 80 min for 24.0, 5.0 and 1.0 h incubation time, followed by a rapid decrease after 80 min indicating a drop in toxicity. Same phenomena occurred with Na_2SO_4 in which the rate of inhibition increased early until maximum values of 65, 60 and 43% at 24.0, 5.0 and 1.0 h, respectively, then steadily dropped after 20 min. Of course, the toxicity of CBZ after treatment relates strongly to the by-products produced during electrochemical



Scheme 1. Electrochemical degradation pathway proposed for CBZ (a) in the presence of NaCl and (b) in the presence of Na_2SO_4.

oxidation treatment. However, Figure 5a shows the appearance and disappearance of the by-products (**I**, **II**, **III**, **IV**, **V** and **VI**) which is probably responsible for the inhibition of Luminous *E. coli* bacteria. Product **I** reached early to its maximum at 20 min then rapidly decreased to the minimum value. There seems to be no effect on the toxicity since the toxicity still increase with decreasing product **I**. Three products (**II**, **IV** and **VI**) are the most effective on the inhibition of Luminous *E. coli* bacteria. The highest value of the three products is at 80 min which could be responsible for the highest value of the toxicity at 80 min. This behavior was also reported by some authors for different compounds.[33,34] From Figure 5b, it was observed that the inhibition of Luminous *E. coli* bacteria was increased to 60% after 20 min of treatment

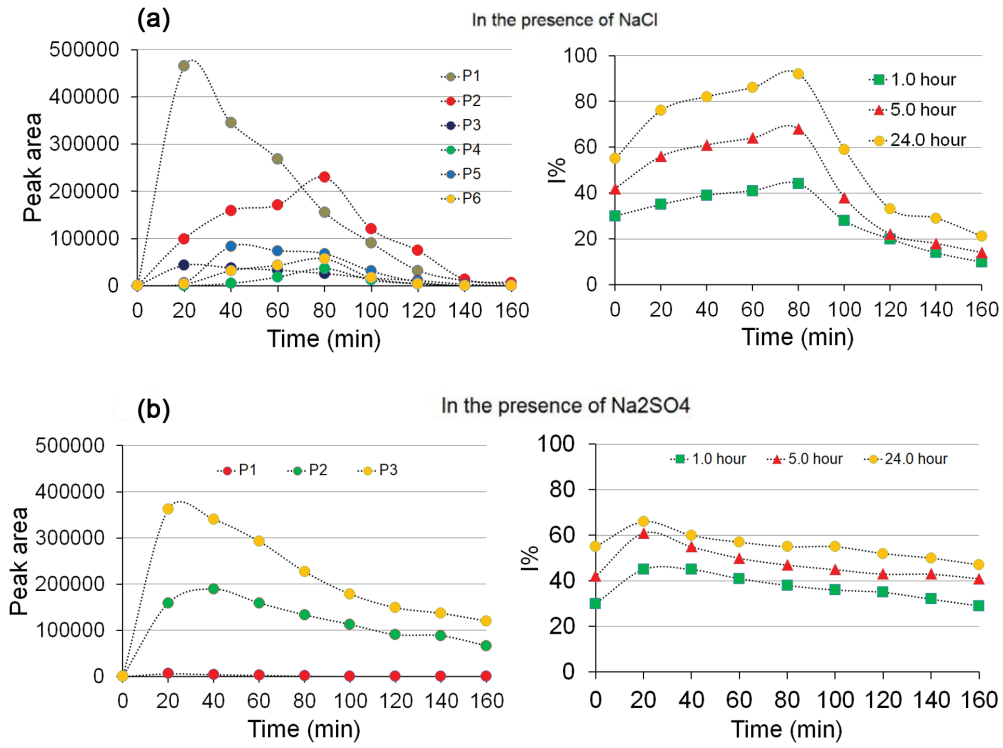


Figure 5. Evolution of toxicity to *E. coli* bacteria after 1.0, 5.0 and 24.0 h of exposure (a) in the presence of NaCl and (b) in the presence of Na_2SO_4. The evolution of the by-products during electrochemical degradation of CBZ is depicted in each case. Experimental conditions: $[CBZ]_0 = 5$ mg L^{-1}; 0.2 g NaCl; and 0.2 g Na_2SO_4.

and 24.0 h of incubation. The reason may be attributed to the formation of three effective products (**VII**, **VIII** and **XI**) in the presence of Na_2SO_4 at the same time of treatment, 20 min. This value is still much lower than 90% (in the presence of NaCl), which could indicate formation of non-chlorinated by-products (less toxicity) as compared to the chlorinated by-products. From these results, NaCl is considered a more suitable electrolyte since all by-products disappeared after 140 min compared to those formed with Na_2SO_4 which is still accumulated in solution. In contract, the toxicity of the carbamazepine and its by-products is almost vanished after 120 min to very low value of 15%, which is lower than the initial toxicity of original compound of 30%.

Conclusions

Electrochemical oxidation process of carbamazepine in water sample was carried out in this work. The effect of various operating parameters such as amount of NaCl, initial concentration of carbamazepine, applied voltage and treatment time was designed by using 2^4 full factorial design on removal efficiency (%) of CBZ. According to the factorial design plots, the most significant factors on removal efficiency (%) were applied voltage (C), NaCl amount (A) and treatment time (D). However, the interaction factors AC and CD were also significant. Conversely, the other interactions were not more significant like ABC, AB and ABD.

The formation of by-products was presented and elucidated using very advanced and accurate instrument (LC-TOF/MS). Six by-products were analyzed and identified correctly in the presence of NaCl while three by-products were only analyzed and identified in the presence of Na_2SO_4. Chloro-epoxy carbamazepine was identified for the first time after electrochemical treatment for carbamazepine. NaCl electrolyte was considered more effective compared to Na_2SO_4 in terms of degradation efficiency of CBZ. Hypochlorite OCl^- was observed as a powerful oxidizing agent to reduce the toxicity of CBZ and its by-products. However, in this study the toxicity was evaluated at different incubation times (1.0, 5.0 and 24.0 h) against the by-products which are formed during electrochemical oxidation process of carbamazepine. The toxicity of the treated carbamazepine solution samples was assessed by measuring the inhibition of *E. coli* bacteria.

Supplementary Information

Supplementary information (scheme for making graphite-PVC composite pellet) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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