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Photocatalytic degradation of psychoactive drugs Alprazolam, Bromazepam and Diazepam by PhotoFenton

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Abstract

Photo-Fenton has emerged as a prominent strategy for the treatment of drugs and their metabolites. Benzodiazepines and their metabolites represent a significant group of emerging environmental pollutants due to their stability, continuous discharge in the environment and their low removal rates by conventional biological wastewater treatment plants. The objectives of the study were a) to optimize the efficiency of the photo catalytic oxidation of three psychiatric pharmaceuticals Alprazolam, Bromazepam and Diazepam using response surface methodology, b) to evaluate the degradation kinetics of the Photo Fenton reaction and c) to examine the effect of various parameters that affect the efficiency and the rate of the oxidative process. To evaluate the degradation rates of the three drugs, a central composite design was selected in order to observe the effects of Fe^{2+} and H_2O_2. Different H_2O_2: Fe^{2+} molar ratios were examined for their kinetic behavior, which was described effectively by pseudo first order kinetic models. The impact of the factors affecting the rates of oxidation of the three drugs such as different iron sources and initial drug concentrations was also investigated.

Keywords: Benzodiazepines, Photo Fenton, Central Composite Design

1. Introduction

The presence of various pharmaceuticals in the environment has been of great interest during the past few years. Urban wastewater and sewage treatment plants have been pointed out as the main source of their discharge into the environment, since conventional treatment fails to completely eliminate them (Gros *et al*., 2010; Oulton *et al*., 2010). Surprisingly, there is still a significant lack of knowledge of their transport and fate in the environment, their degradation and the degradation products formed. Pharmaceuticals and drugs of abuse have been widely detected in many environment compartments, especially in the aquatic one: many studies in Europe and the USA highlight the multitude of active pharmaceutical principles which are present in waste, surface, ground and drinking water (Bueno *et al*., 2010; Mendoza *et al*., 2014a; Mendoza *et al*., 2014b; Ternes *et al*., 2001)

Benzodiazepines compose a class of drugs with anticonvulsant, hypnotic and anxiolytic properties and have been established as the selected drug for the treatment of disorders due to anxiety and insomnia. They are among the most prescribed drugs worldwide, while Europe is the continent with the highest consumption (International Narcotics Control Board, 2009) Benzodiazepines considered to be stable compounds in terms of biodegradation, not fully metabolized by the human body, whereas they are also relatively resistant to degradation methods applied during their presence in sewage treatment plants(Redshaw *et al*., 2008). Photo Fenton reaction have been demonstrated to be a suitable oxidation method for the removal of organic compounds because highly oxidizing reactive oxygen species i.e. hydroxyl radicals (•OH) are catalytically produced. During the catalytic reaction, ferrous ions are continuously regenerated enhancing the efficiency of the process (Mirzaei *et al*., 2017; Trawiński and Skibiński, 2017). Apart from being an efficient treatment technique, Fenton oxidation could also serve as an adequate pretreatment method followed by biodegradation and has been proven to reduce toxicity of the pharmaceutical wastewaters. (Tekin *et al*., 2006) Response surface methodology (RSM) is a chemometric approach that has been a useful tool during the last years for optimizing experimental methodologies with a reduced number of experiments, lower cost and the additional possibility to evaluate interactions among variables. It is a collection of mathematical and statistical techniques based on the fit of a polynomial equation (model) to the experimental data, which could describe the behavior of a data set with the objective of making statistical previsions. These techniques also enable the selection of optimal experimental conditions, helping to avoid trivial mistakes during optimization (Leardi, 2009; Speck *et al*., 2016). The objectives of the study were a) to optimize the efficiency of the photocatalytic oxidation of three psychiatric pharmaceuticals i.e., Alprazolam, Bromazepam and Diazepam through RSM, b) to evaluate the kinetics of the Photo Fenton reaction under different experimental conditions and c) to examine the effect of various parameters on the efficiency and the rate of the oxidative process.

2. Materials And Methods

a. Reagents

All chemicals were of analytical grade. The selected drugs Alprazolam, Bromazepam and Diazepam were used in solid crystalline form, purity 99.9% by Lipomed. $Fe_2(SO_4)_3$, H_2SO_4, H_2O_2 30% w/v were purchased from the company Panreac

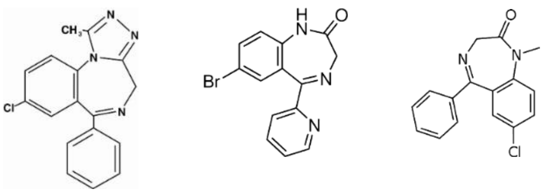
Alprazolam **Bromazepam** **Diazepam**

Figure 1. Structures of the selected psychoactive drugs

2.2. Kinetic experiments

The experiments were conducted at ambient temperature (25 ± 3^{0}C) and under magnetic stirring in a photolysis device equipped with a high pressure mercury lamp HPK 125 W Cathode on (radiant flux 55mW cm-2). The lamp was surrounded by a Pyrex filter, to isolate wavelengths smaller than 290 nm. 500mL of active substance solutions were added with the initial concentration of 10mg L^{-1} for diazepam, 10mg L^{-1} for Alprazolam and 12mg L^{-1} for Bromazepam. Initially, a pH adjustment was made to the appropriate value, i.e., 2.9 ± 1 with the corresponding acid, depending on the salt used as the source of iron ions. After adjusting the pH, the necessary amounts of iron salt solution and hydrogen peroxide were added to the system. Periodically, samples were withdrawn in order to measure the concentration of the active substance, the concentration of residual peroxide, the total organic carbon (TOC), as well as concentration levels of different iron forms which remain in the solution (both the divalent and the total iron). The concentration of Alprazolam, Bromazepam and Diazepam was determined with a high-pressure liquid chromatography on a water BEH C18 chromatography column 1.7mm, 2.1x 100mm coupled with a PDA detector.

2.3. Optimization of drug degradation

A central composite design (CCD) was carried out considering the minimum and maximum levels for H_2O_2 concentration (10-60mg L^{-1}) and Fe^{3+} concentration (1-10mg L^{-1}) as it is shown in Figure 2. It is noteworthy that the ranges considered for the two studied variables were chosen based on literature findings as well as in our experiments previously performed in the laboratory. The objective function was to study the kinetics of the Photo Fenton reaction, explore the relationship between dependent variables and the output and optimize the reduction of the selected drugs especially at 3min of the reaction. The experiments were arranged according to an experimental design to reveal the effect of H_2O_2 and Fe^{2+} concentration through mathematical models. The significance of each model was identified using the value p-value ($p < 0.05$) for a confidence level of 95% and the null assumption that the current model is not important.

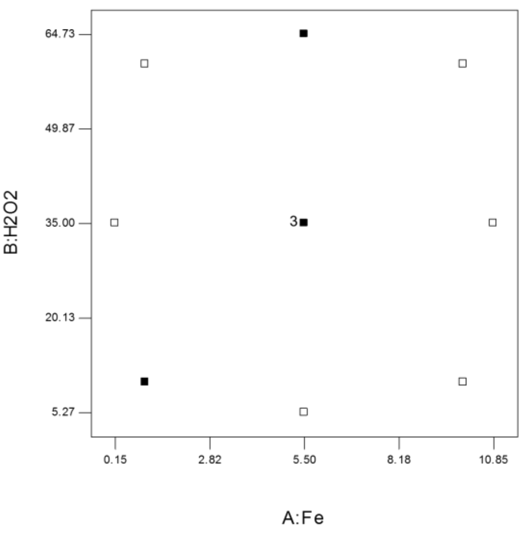

Figure 2. Concentration ranges for ferric iron ions and hydrogen peroxide

The types of mathematical models that were tested were linear, quadratic and cubic. The accuracy of the mathematical models was evaluated through various parameters, such as the linear correlation coefficient R^2, the linear correction coefficient R^2_{Adj} and the relative standard deviation CV%. The statistical analysis was performed using ANOVA from the Design Expert statistical software 7.0.0 (StatEase Inc., Minneapolis, USA).

3. Results And Discussion

Table 1 shows all these statistical parameters for each of the selected mathematical models describing the photocatalytic degradation of the three compounds.The greatest influence on the selected model corresponds to the change of ferric iron and peroxide concentrations. Figures 3-5 show the effect of peroxide and ferric ion concentration on the kinetics of the oxidative degradation of Alprazolam, Bromazepam and Diazepam for the first 3 min of the reaction. It was generally observed that as the concentration of the two variables increase, the degradation of each studied compound in the first three minutes of the experimental process becomes faster, reaching a certain maximum and decreasing again. Experimental values of pseudo order reaction constants ranged from 0.023 to 0.367min^{-1}, 0.036 to 0.447 min^{-1} and 0.063 to 0.890 min^{-1} for Alprazolam, Bromazepam and Diazepam, respectively. This behaviour is expected because according to the mechanism of reaction Photo Fenton increasing the initial concentration of Fe^{3+} leads to higher amount of generated hydroxyl radicals, which is the main compound of oxidation. The effect of increasing concentrations of independent variables in the model response is verified by the sharp rise of the curves in the red region of interaction diagram especially in the degradation of Alprazolam and Diazepam. The best performance of the system occurs when the experimental values of the concentration of ferric sulphate is greater than 5.5 mg L^{-1}. At higher iron concentrations though, the excess of the oxidative radicals appear to be quite large and a further increase in their concentration will not contribute significantly to an increase in the rate of oxidation reaction. Proportionally, at peroxide concentration higher than 35 mg L^{-1} the limitation in the increase on oxidation rate is attributed to the known property of H_2O_2 acting as a scavenger of the hydroxyl radicals according to the following reactions

$$H_2O_2 + OH^{\bullet} \rightarrow H_2O + HO_2^{\bullet}$$

$$HO_2^{\bullet} + OH^{\bullet} \rightarrow H_2O + O_2$$

$$OH^{\bullet} + OH^{\bullet} \rightarrow H_2O_2$$

More specifically, the model describing the effect of various parameters on the pseudo first-order reaction constant of Alprazolam for the first three minutes of the reaction is given in table 1. The significant parameters were A (Fe), B (H_2O_2), A^2 $(Fe)^2$ and B^2 $(H_2O_2)^2$ with p-value 0.0043, 0.0071, 0.0008 και 0.0061, respectively.

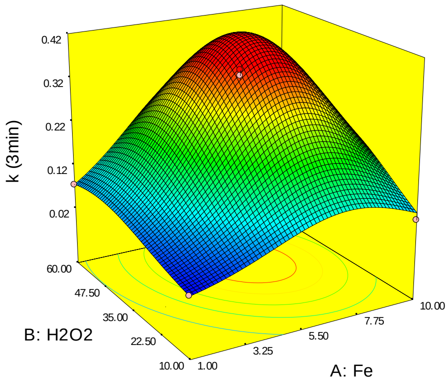

Figure 3. Response surface and level curve corresponding to the kinetics of degradation of Alprazolam the first three minutes of the reaction as a function of Fe^{3+} and H_2O_2 concentration

The models describing the effect of various parameters on the pseudo first-order reaction constant of Bromazepam and Alprazolam, in the first 3 min of reaction, are given in table 1.

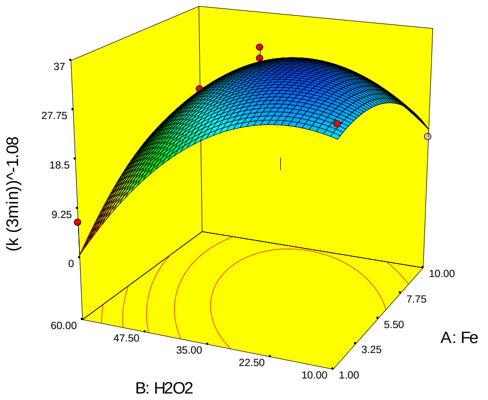

Figure 4. Response surface and level curve corresponding to the kinetics of degradation of Bromazepam the first three minutes of the reaction as a function of Fe^{3+} and H_2O_2 concentration

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Table 1. Mathematical models describing the effect of selected parameters on the degradation kinetics for Alprazolam, Bromazepam and Diazepam (3 min reaction time) and statistical parameters for the evaluation of the models

Compound	**Model**	**P value (model significance)**	**p value (Lack-of-fit)**	**R^2**	**R^2 adj**	**Std. Dev.**	**C.V. %**
Alprazolam	Quadratic $k_{3min}^{-0.15} = 2.09470 - 0.15370*Fe - 0.021341*H_2O_2 + 4.59140^{E-004}*Fe*H_2O_2 + 0.010336*Fe^2 + 2.14208^{E-004}*H_2O_2^2$	0.0018	0.1257	0.958	0.916	0.05	4.12
Bromazepam	Quadratic $k_{3min}^{-1.08} = 23.51450 + 1.14465*Fe + 0.56971*H_2O_2 + 0.063990*Fe*H_2O_2 - 0.28478*Fe^2 - 0.017181*H_2O_2^2$	0.0283	0.1436	0.870	0.741	5.67	25.59
Diazepam	Quadratic $k_{3min}^{-0.64} = 12.57061 - 1.47130*Fe - 0.27341*H_2O_2 + 2.75522E^{-003}*Fe*H_2O_2 + 0.084578*Fe^2 + 2.71754E^{-003}*H_2O_2^2$	0.0004	0.0901	0.977	0.955	0.55	16.1

For Diazepam, the significant parameters are A (Fe^{3+}), B(H_2O_2), A^2 (Fe^{3+})^2 and B^2 (H_2O_2)^2 with p-value 0.0002 values, 0.0005, 0.0019 and 0.0020, respectively.

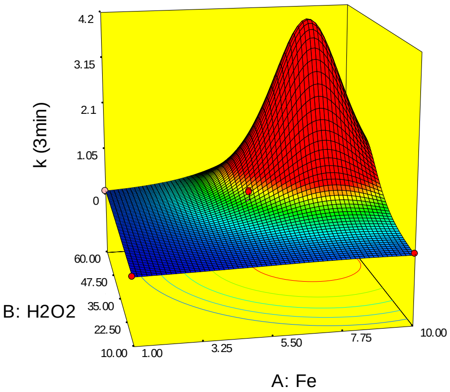

Figure 5. Response surface and level curve corresponding to the kinetics of degradation of Diazepam the first three minutes of the reaction as a function of Fe^{3+} and H_2O_2 concentration

Table 2. The concentrations of $Fe_2(SO_4)_3$ and H_2O_2 for each pharmaceutical for the fast and environmentally friendly scenario

	Fast scenario			**Environmentally friendly scenario**		
	Fe^{3+}(mg/L^{-1})	**H_2O_2 (mg/L^{-1})**	**k (3min)**	**Fe^{3+}(mg/L^{-1})**	**H_2O_2 (mg/L^{-1})**	**k (3min)**
Alprazolam	6.39	45.67	0.42	2.96	46.65	0.209
Bromazepam	4.61	25.08	0.11	1.00	18.41	30.22
Diazepam	7.49	55.42	2.04	2.9	48.86	0.234

Based on the mathematical models describing the experimental data, two sets of optimised values for the experimental parameters were proposed, providing a) the highest reaction rate constant (fast scenario) and b) the highest reaction rate requiring minimum initial iron concentration (environmentally friendly scenario) (Table 2).The experiments were performed under the optimised values for the "environmentally friendly" scenario (Table 2). Results (Figure 6) indicated that Alprazolam was reduced to half during the first 30 minutes of reaction, while 72.04% was degraded at 60 minutes and 95.69% at 180 minutes, respectively. At 180 min, 95.7% of Alprazolam and 71.3% of Bromazepam was removed, respectively. Bromazepam showed the slowest degradation percentages; 31.50% at 30 minutes, 42.54% at 60 minutes and 71.37% at 180 minutes respectively.

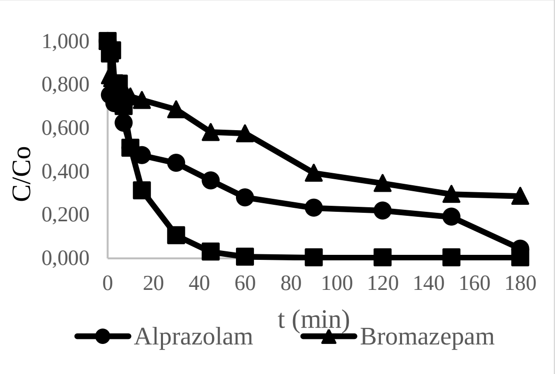

Figure 6. Degradation of Alprazolam, Bromazepam and Diazepam under Photo Fenton oxidation: Alprazolam, Co = 10mg L^{-1} Fe^{3+}=2.96 mg L^{-1} H_2O_2 = 46.65mg L^{-1} / Bromazepam, Co = 12mg L^{-1} Fe^{3+}=1.00 mg L^{-1} H_2O_2 = 18.41 mg L^{-1} / Diazepam Co = 10mg L^{-1} Fe^{3+}=2.90 mg L^{-1} H_2O_2 = 48.86mg L^{-1}

4. Conclusions

The degradation of the selected benzodiazepines Alprazolam, Bromazepam and Diazepam proved to be effective with a Photo Fenton system optimised using Response Surface Methodology. Full degradation of alprazolam and diazepam was achieved under 180 min of reaction time. Two sets of experimental conditions were proposed, taking into consideration criteria related to the reaction rate and the minimum use of iron.

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