Environ. Eng. Res. Vol. 14, No. 2, pp. 81~87, 2009
Korean Society of Environmental Engineers

Decomposition of Antibiotics (Cefaclor) by Ionizing Radiation: Optimization and Modeling Using a Design of Experiment (DOE) Based on Statistical Analysis

Seungho Yu[1], Soon-Woong Chang, Si Jin Lee, and Il-Hyoung Cho†

Department of Environmental Engineering, Kyonggi University, Suwon 443-760, Korea
[1]Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup 580-185, Korea

Received May 2008, accepted April 2009

Abstract

The decomposition of antibiotics (cefaclor) by gamma irradiation in aqueous solutions was experimentally evaluated. To obtain a mutual interaction between two factors (antibiotics concentrations and radiation doses) and to optimize these factors during the process, experimental design and statistical analysis were employed. The decomposition capability of the gamma radiation was also mathematically described as a function of cefaclor concentration and gamma-ray dose using the statistical analysis. The results showed that the cefaclor concentration (X_1) in the response Y_1 (Reduction of cefaclor concentration) and gamma-ray dose (X_2) in the response Y_2 (Removal efficiency (%) of cefaclor concentration) exhibited a significantly positive effect, whereas gamma-ray dose (X_2) in the response Y_1 showed a significantly negative effect. The estimated ridge of maximum responses and optimal conditions for Y_1: (X_1, X_2) = (25 mg/L, 350 Gy) and Y_2: (X_1, X_2) = (21 mg/L, 565 Gy) using canonical analysis were 4.37 mg/L of reduction of cefaclor concentration and 98.35% of removal efficiency of cefaclor concentration, respectively. The measurement values agreed well with the predicted ones, thereby confirming the suitability of the model for Y_1 and Y_2 and the success of the experimental design in optimizing the conditions of the gamma irradiation process.

Keywords: Antibiotics, Cefaclor, Gamma radiation, Experimental design, Statistical analysis

1. Introduction

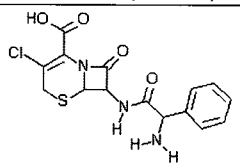
Recently, a number of investigations have been reported on the widespread occurrence of pharmaceutical compounds in the environment, notably in the aquatic compartment, thus establishing these compounds as a new class of pollutants.[1)] Recent studies have revealed the detection in various environments of antibiotics to control bacteria in humans and animals.[2,3)] Antibiotics used for humans and animals can be introduced to different environmental compartments as parent compounds or metabolites, mainly by an excretion, improper disposal of expired or surplus medications into sewage systems, and agricultural and aquacultural activities.[4,5)] Accordingly, a variety of antibiotics and their metabolites have been found in municipal wastewater treatment plants, surface water, and groundwater.[6-9)]

Optimization for the treatment process of environmental pollutants involves changing one independent variable while holding all others constant. This is extremely time-consuming and expensive for an large number of variables and yet may produce inaccurate conclusions.[10)] Response surface methodology (RSM), an experimental strategy for seeking the optimum conditions for a multivariable system, is an efficient technique for optimization. Recently, it has been successfully applied to the optimization of an antibiotic process for using experimental designs, which include optimization of medium composition for actinorhodin production,[11)] bacteriocin production by *Bacillus licheniformis* strain P40 in cheese[12)] and simultaneous determination of tetracyclines in pharmaceuticals by capillary zone electrophoresis.[13)] However, no study has reported on the treatment efficiency and the interaction effect of various parameters using experimental design methodology during the ionizing radiation process for decomposition of antibiotics.

Among the many kinds of antibiotic, the target antibiotic compound selected in this study was cefaclor (Table 1), a β-lactam antibiotic that is widely used in the medical treatment of microbial infectious diseases caused by bacteria such as pneumonia and infections of the ear, lung, skin, throat, and urinary tract.[14)]

† Corresponding author
E-mail: cihyoung73@hanmail.net
Tel: +82-31-255-9739, Fax: +82-31-254-4905

Table 1. Chemical structure of dyes and the properties of antibiotics (cefaclor)

No	antibiotics (cefaclor)
Chemical structure	
Molecular formula	$C_{15}H_{14}ClN_3O_4S$
M.W	367.81 g/mol
Water solubility	8.6 g/L
Class	ß-lactam

Cefaclor is classified into cephalosporins and has the same fundamental structure as penicillin. Moreover, cefaclor is one of the most prescribed antibiotics in many countries, and it is also detected at several μg/L in sewage treatment plants.[15,16]

One is often interested in finding a suitable approximating function for the purpose of predicting and determining the future response. Experiment of design (DOE) based on the statistical analysis are not only primarily used for the purpose of allowing the researcher in order to understand the mechanism of the system or process; rather its purpose is to determine the optimum operating conditions or to determine a region for the factors at a certain operating specification. In this work, the gamma radiation process was applied for the decomposition of cefaclor in water. Experimental design was employed for the modeling and optimization for the degradation condition of cefaclor using statistical analysis.

2. Materials and Methods

Cefaclor was obtained from Sigma-Aldrich, Co. (St Louis, MO, USA). Cefaclor concentrations in the aqueous samples were determined by using high performance liquid chromatography (HPLC). An Agilent 1200 Series HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a UV absorbance detector was operated at 254 nm. Separation of cefaclor was achieved by using a Phenomenex Synergi 4μ Polar-RP column (150 × 4.6 mm) with 20 mM ammonium formate adjusted to pH 3.5/methanol (65:35 ratio) as the eluent at a flow rate of 1.0 mL/min. Five- or six-point linear standard calibration curves were constructed before and periodically throughout the analyses period to verify the system stability. Duplicate or triplicate samples were prepared and analyzed for each sample.

Gamma irradiations were carried out with a high-level ^{60}CO source (Nordion Inc., Canada) at the Korea Atomic Energy Research Institute (Jeongeup, Korea). The radioactivity of the source was around 1.47×10^{17} Bq (= 397949 Ci), and the dose rates ranged from 6.3 to 14.3 kGy/hr depending on the distance from the source (up to 100 kGy). The absorbed doses were measured by the alanine-EPR dosimetry system (ISO/ASTM 51607:2003). For gamma radiolysis, the aqueous sample solutions containing cefaclor were placed into 125 mL glass screw cap bottles without a headspace. All the solutions were in equilibrium at atmospheric pressure and room temperature (22°C±2) before irradiation and were sealed with screw caps to prevent air contamination.

3. Results and Discussion

3.1. Decomposition of Cefaclor by Gamma Irradiation

The effect of initial cefaclor concentration and initial gamma-ray dose on the degradation was studied by varying the cefaclor concentration (factor X_1) and gamma-ray dose (X_2), as shown in Table 2. Experiments were conducted in quintuplicate to ensure the reproducibility of the results for Y_1 (change of cefaclor concentration) and Y_2 (gamma-ray dose) for each cefaclor concentration and gamma-ray dose tested in this study. The aqueous concentration of cefaclor irradiation ranged from 9 to 30 mg/L at factor X_1. The irradiation dose (X_2) ranged from 0 to 700 Gy. As shown in Table 2, the reduction of cefaclor concentration decreased with increasing absorbed dose. Cefaclor at a concentration of 9 to 30 mg/L was almost degraded between 300 and 700 Gy, respectively.

Table 2. The design of experiment and response (Y_1, Y_2) for decomposition of cefaclor

Run	Factor (variable)		Measured data		Predicted data	
	X_1[a]	X_2[b]	Y_1[c]	Y_2[d]	Y_1	Y_2
1	9	0	8.2	0.0	7.93	0.2
2	9	100	2.7	67.2	3.89	53.4
3	9	200	0.8	90.0	0.98	80.8
4	9	300	0.3	96.8	-0.8	102
5	13	0	12.0	0.0	6.44	1.1
6	13	100	5.1	57.7	2.98	44.4
7	13	200	2.4	80.3	0.65	91.6
8	13	300	0.9	92.2	-0.55	96.1
9	13	400	0.4	96.9	16.21	98.4
10	20	0	17.5	0.0	10.8	4.59
11	20	100	10.2	41.6	6.50	60.9
12	20	200	5.8	66.8	3.34	80.5
13	20	300	3.6	79.6	1.31	94.6
14	20	400	2.0	88.8	0.40	100.3
15	20	500	0.9	94.6	0.63	100.5
16	20	600	0.6	96.6	1.98	94.3
17	20	700	0.5	97.3	25.1	-1.05
18	30	0	26.1	0.0	18.4	1.53
19	30	100	17.2	34.3	12.9	34.9
20	30	200	12.1	53.5	8.50	53.2
21	30	300	8.2	68.6	5.18	85.1
22	30	400	5.2	80.0	3.03	90.5
23	30	500	3.5	86.8	2.02	89.5
24	30	600	2.6	90.1	2.13	82.0

[a]Cefaclor concentration (mg/L), [b]Gamma-ray dose (Gy), [c]Reduction of cefaclor concentration (mg/L), [d]Removal efficiency (%) of cefaclor concentration

The data in Table 2 indicate the influential role of the investigated factors (concentration and irradiation dose) on the degradation percentages of cefaclor (Y_2 in measure data). In this respect, the degradation percentage increased with increasing irra-

diation dose. This was observed over all tested concentrations, especially at the lowest targeted concentration (9 mg/L), where the degradation percentages were 67%, 90% and 96.8% for absorbed doses of 0.1, 0.2 and 0.3 kGy, respectively. These results also indicated that most of the cefaclor concentration was degraded in solution when exposed to different doses of high-energy irradiation (runs 4, 8, 9, 15, 16, 17 and 24 in Table 2). The cefaclor destruction was explained as follows. The primary oxidation steps during gamma radiolysis in the aqueous solutions are induced by ·OH radicals. Upon radiation absorption in water via gross reaction (1), approximately equal amounts of reducing and oxidizing species are formed ($G(\cdot OH) \approx G(eaq^-) \approx 2.9\times 10^{-7}$ mol $dm^{-3}J^{-1}$, $G(H\cdot)=0.6\times10^{-7}$ mol $dm^{-3}J^{-1}$, $G(H_2O_2)=0.7\times 10^{-7}$ mol $dm^{-3}J^{-1}$). In the presence of air, with an oxygen partial pressure of $[O_2]=0.25\times10^{-3}$ mol dm^{-3}, the reducing radicals H· and e_{aq}^- are converted into $HO_2\cdot$ and $O_2^{\cdot-}$, (reactions (2)-(4)). The superoxide radicals exhibit low reactivity towards aromatic acids and phenols, and instead decay mainly by combination via reaction (5).[17-19]

$$H_2O \text{—} \wedge \vee \rightarrow {}^{\bullet}H, e_{aq}^-, {}^{\bullet}OH, H_2, H_2O_2, H_3O^+, OH^- \quad (1)$$

$$H^{\bullet} + O_2 \rightarrow HO_2^{\bullet} \quad k = 2.1 \times 10^{10}\ dm^3\ mol^{-1}s^{-1}, \quad (2)$$

$$e_{aq}^- + O_2 \rightarrow O_2^{\bullet-} \quad k = 2.0 \times 10^{10}\ dm^3\ mol^{-1}s^{-1}, \quad (3)$$

$$H^+ + O_2^{\bullet-} \leftrightarrow HO_2^{\bullet} \quad pK = 4.8, \quad (4)$$

$$HO_2^{\bullet} + O_2^{\bullet-} \rightarrow HO_2^- + O_2 \quad k = 1.02 \times 10^8\ dm^3\ mol^{-1}s^{-1}. \quad (5)$$

3.2. Estimation of the Multi-regression Model and Verification

This multivariate approach was performed with a cefaclor concentration range of 9~30 mg/L and a gamma-ray dose range of 0-600Gy. To estimate the coefficients in the approximating polynomial function by applying the values of factor levels, the least squares regression method was performed using the Minitab Release 14 and SAS System statistical software (Release 8.2). In order to optimize the gamma-ray activity of cefaclor decomposition, an experimental design was carried out following the methodology described by Box.[20] The measured response was defined as the change of cefaclor concentration (Y_1) and removal efficiency (%) of cefaclor concentration (Y_2) (Table 2). A quadratic model, which also includes the linear model, is given.[20]

$$Y = b_o + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k-1}\sum_{j=2}^{k} b_{ij} x_i x_j + \sum_{i=1}^{k} b_{ii} x_i^2 + e_i \quad (6)$$

where Y is the predicted response, b_0 is the offset term (constant coefficient), b_i is the linear effect, b_{ij} is the squared effect, b_{ii} is the interaction effect, and e_i is the error. Multiple regressions enable a description of the mathematical relationship between the variables and the experimentally obtained responses. The resulting second-degree model in this study is described by the polynomial expression shown in Eq. (7):

$$Y = b_o + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 + e \quad (7)$$

The coefficients b_i and b_{ii} of this model were then calculated in order to obtain the expressions shown in Table 3. The quality of the fit of polynomial model was expressed by the coefficient of determination R^2 and R^2_{adj} in Eqs. (8) and (9), respectively (Körbahti and Rauf, 2007). In the above equations $SS_{effect(model)}$ in Eq. (8-9) is called the sum of square of factor effect and $SS_{error(residual)}$ in Eq. (8-9) represents the sum of squares of error.

Table 3. Regression equations obtained for response Y_1 and response Y_2 of cefaclor

Prediction model based on the first and second order	
Response 1[a]	$R^2 = 98.5\%$, Adjusted $R^2 = 98.1\%$ $Y_1 = 2.46 + 0.56X_1 - 0.035X_2 + 0.0066X_1^2 + 0.00006X_2^2 - 0.00124X_1X_2$
Response 2[b]	$R^2 = 94.7\%$, Adjusted $R^2 = 93.3\%$ $Y_2 = 43.3 - 3.17X_1 + 0.38X_2 + 0.0564X_1^2 - 0.0003X_2^2 - 0.0013X_1X_2$

[a]Reduction of cefaclor concentration (mg/L), [b]Removal efficiency (%) of cefaclor concentration

$$R^2 = 1 - \frac{SS_{residual}}{SS_{model} + SS_{residual}} \quad (8)$$

$$R^2_{asj} = 1 - \frac{SS_{residual}/DF_{residual}}{(SS_{model} + SS_{residual})/(DF_{model} + DF_{residual})} \quad (9)$$

Calculation of the multiple correlation coefficients (R^2) indicated that 98.5% (Adjusted R^2 = 98.1%) and 94.7% (Adjusted R^2 = 93.3%) of the variation of cefaclor concentration and cefaclor removal (%) could be explained using the model polynomial function Y_1 and Y_2, respectively. Actual (observed) versus predicted values were displayed as the real responses data plotted against the predicted responses, and are shown in Fig. 1(a) and (b). The plot showed a satisfactory correlation between the values of experimental data and predictive values. The validation of models was also checked by using graphical residual analysis. The residuals were calculated from the following equation:

$$\text{Residuals} = Y_{i,obser} - Y_{i,pred} \quad (10)$$

In Fig. 2(a) and (b), normal graph of residuals was given. As can be seen all residuals lie on a straight line which shows that residuals were distributed normally. But only one residual deviated from the straight line. The normal probability plot of the student residuals indicated that none of the individual residuals exceeded the residual variance and further confirmed the excellent adequacy of the regression model. These results further proved the good agreement of the experimental values with the predicted values.

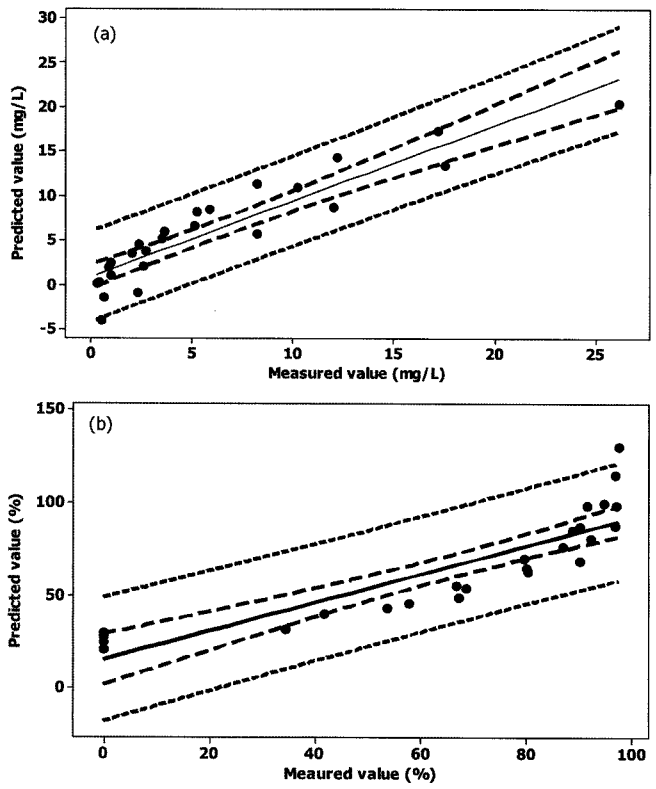


Fig. 1. Plots of observed vs. predicted values derived from the Model of Y_1 (cefaclor conc, mg/L) (a) and Y_2 (removal efficiency (%) (b). The fitted (solid) line based on 95% of confidence interval (long dash line) and 95% of predict interval (short dash line) is the regression line with the coefficient of determination R^2 = 98.5% (a), 94.7% (b). The each point refers to the experiment under listed in Table 2.

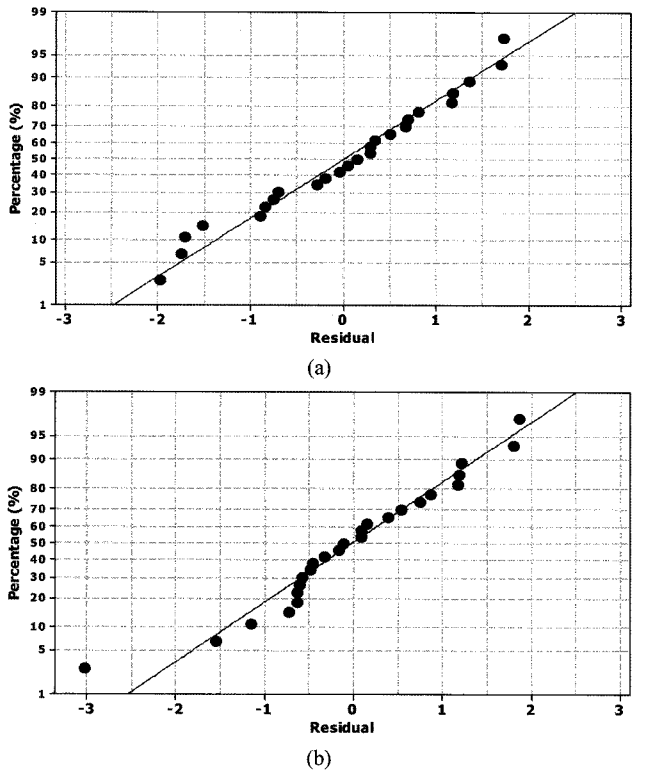


Fig. 2. Residual plot of model for error values; (a) Y_1 (cefaclor conc, mg/L) and (b) Y_2 (removal efficiency (%)).

3.3. Analysis of Variance (ANOVA)

The method used to compare the magnitude of estimated effects of factors with the magnitude of experimental error is called analysis of variance (ANOVA). If the magnitude of a factor effect is large when compared with experimental error, it is decided that the changes in the selected response cannot occur by chance and those changes in the response can be considered to the effects of the factors. The factors causing a variation in the response are called significant. In this study, Fisher's F-test in Eq. (11) was used in the analysis of variance, the terms SS and DF corresponds to sum of squares and degrees of freedom (DF), respectively.

$$F = \frac{\mathrm{MSS}_{\mathrm{effect(model)}}}{\mathrm{MSS}_{\mathrm{error(residual)}}} \tag{11}$$

$$M_{SSeffect(model)} = \frac{\mathrm{SS}_{\mathrm{effect(model)}}}{\mathrm{DF.1}} \tag{12}$$

$$MSS_{error(residual)} = \frac{\mathrm{SS}_{\mathrm{error(residual)}}}{\mathrm{DF.2}} \tag{13}$$

Table 4 showed the results of the quadratic RSM model fitting in the form of analysis of variance (ANOVA). ANOVA is required to test the significance and adequacy of the model. The mean squares (MS) are obtained by dividing the sum of the squares (SS) of each of the two sources of variation, the model and the error variance, by the respective degrees of freedom (DF). The Fisher variation ratio, the F-value (=Sr^2/Se^2), is a statistically valid measure of how well the factors describe the variation in the data about its mean square due to model variation by that due to error variance. Increasing distance of the F-value from unity indicates increasing certainty that the factors adequately explain the variation in the data about their mean, and that the estimated factor effects are real.

Using the 1% significance level, a model is considered significant if the p-value (significance probability value) is less than 0.01. The ANOVA results of the quadratic regression model demonstrated the high significance of the model, as further confirmed by the F-test (F_{model} = 242.9 for response Y_1, F_{model} = 68.02 for response Y_2) results and the very low p-value (p<0.0001

Table 4. Analysis of variance (ANOVA) for two reponses (Y_1, Y_2)

Source of variation	Responses			
	Y_1		Y_2	
	F value	p-value	F value	p-value
Model	242.9	<0.0001[a]	68.02	<0.0001
Linear contribution	46.60	<0.0001	58.69	<0.0001
Quadratic contribution	81.22	<0.0001	32.77	<0.0001
Cross-product contribution	67.98	<0.0001	0.75	0.396

[a]Significant at 1% (p-value)

for responses Y_1 and Y_2). From the p-value presented in Table 4, it can be concluded that for both responses, the linear and quadratic contributions of the model were significant ($p<0.0001$). In addition, for response Y_1, all interaction contributions of the quadratic and cross-production of the model were significant ($p<0.0001$), whereas for response Y_2 only the cross-production contribution of the model was significant ($p<0.0001$).

3.4. Estimation of Quantitative Effects of the Factors

The parameter estimation and corresponding p-values (Table 5) suggested that cefaclor concentration (mg/L) and gamma-ray dose (Gy), among the tested variables, exerted the largest effect on the reduction of cefaclor concentration and removal efficiency (%) of cefaclor. Student's t-test was performed to estimate the quantitative effects of the factors. Response surface regression analysis for each factor was performed using the values of the factor levels. Table 5 presents the factor effects of the model and associated p-values for the two responses. A factor was considered to have influenced the response if the effects significantly differed from zero and the p-value was less than 0.01. Positive and negative signs indicated a synergistic and antagonistic effect, respectively, of the factor on the selected response.

As shown in Table 5, the response of Y_1 (reduction of cefaclor concentration) was significantly affected by the synergistic effects of the linear term of cefaclor concentration (X_1) (p-value of 0.003) and the pure quadratic term of gamma-ray dose (X_{22}) (p value of 0.001), as well as by the antagonistic effects of the linear term of gamma-ray dose (X_2) (p value of 0.001) and the cross-product (interaction) term of cefaclor concentration (X_1) × gamma-ray dose (X_2) (p value of 0.001). However, the significant factors for the response Y_2 were the linear term of the gamma-ray dose (X_2) with a p-value of 0.001 on the synergistic effect and the pure quadratic term of the gamma-ray dose (X_{22}) with a p-value of 0.001 on the antagonistic effect.

3.5. Response Surface (contour) Plots and Optimization Conditions

Response surface and contour plots provide a method to predict the reduction and removal efficiency (%) for different values of the tested variables while the contours of the plots help to identify the type of interactions between these variables. Each contour curve represents an infinite number of combinations of the two tested variables with the other two maintained at their respective zero levels. A circular contour of response surfaces indicates a significant interaction between the corresponding variable. In contrast, the saddle nature of the contour plots indicates that the interaction between the corresponding variables is negligible.

Three-dimensional (3-D) and contour (2D) plots for the predicted responses were also formed, based on the model polynomial functions, to assess the change of the response surface. In addition, the relationship between the dependent (Y_1, Y_2) and independent variables (X_1, X_2) can be further elucidated by these plots. Since the model has more than two factors, one factor was held constant for each diagram, leading to the production of 2 response surface diagrams for each response (Y_1, Y_2). Fig. 3(a) and (b), describing the minimum point for the Y_1 response, presents graphical 3D and 2D representations of the polynomial obtained from the matrix. As seen in this figure, the cefaclor concentration (Y_1) is inversely correlated with the cefaclor concentration. As can be seen in Fig. 3(a) and (b), the highest reduction of cefaclor concentration was achieved over a cefaclor concentration range of 0 to 18 mg/L and irradiation dose rang4e of 240 to 570 Gy, in line with the interaction effect between cefaclor concentration (X_1) and gamma-ray dose (X_2). The effect of cefaclor concentration (X_1) and gamma-ray dose (X_2) is also shown in Fig. 4(a) and (b), which presents the maximum point for Y_2 response. This contour plot indicates a wide range of cefaclor removal (%) combinations resulting in a low cefaclor concentration. On the contrary, working at low gamma-ray irradiation values, the cefaclor concentration did not significantly affect the percentage of cefaclor removal. However, the synergistic interaction of cefaclor concentration (0 to 21 mg/L) and gamma-ray dose (300 to 700 Gy) achieved almost complete degradation. These results demonstrated that the reduction (Y_1) and removal efficiency (%) (Y_2) of cefaclor concentration is not dependent on the cefaclor concentration, but is very significantly affected by the gamma-ray dose.

Canonical analysis using the SAS package for optimization conditions is a mathematical approach to examine the over-all shape of the curve, to locate the stationary point of the response surface, and to decide whether it describes a maximum, minimum, or saddle point. The stationary or centroid point is defined as the point at which the slope of the response surface is zero when taken in all directions. The coordinate of this point are termed the stationary point coordinates (optimum point).

Table 5. Factor effects and associated p-values for two responses

Relationship		Responses					
		Y_1			Y_2		
		T value	Effect[d]	p-value[e]	T value	Effect[d]	p-value[e]
Main effects	X_1[a]	3.34	S	0.003	-1.987	A	0.061
	X_2[a]	-9.51	A	0.001	10.83	S	0.001
Interactions	X_1^2[b]	1.52	S	0.144	1.37	S	0.187
	X_2^2[b]	12.7	S	0.001	-7.58	A	0.001
	X_1X_2[c]	-8.25	A	0.001	-0.87	A	0.396

[a]Linear terms, [b]Pure quadratic terms, [c]Cross-product (interaction), [d]Synergistic effct, antagonistic effect, [e]Significant at 1% (p-value), Significant at 5% (p-value)

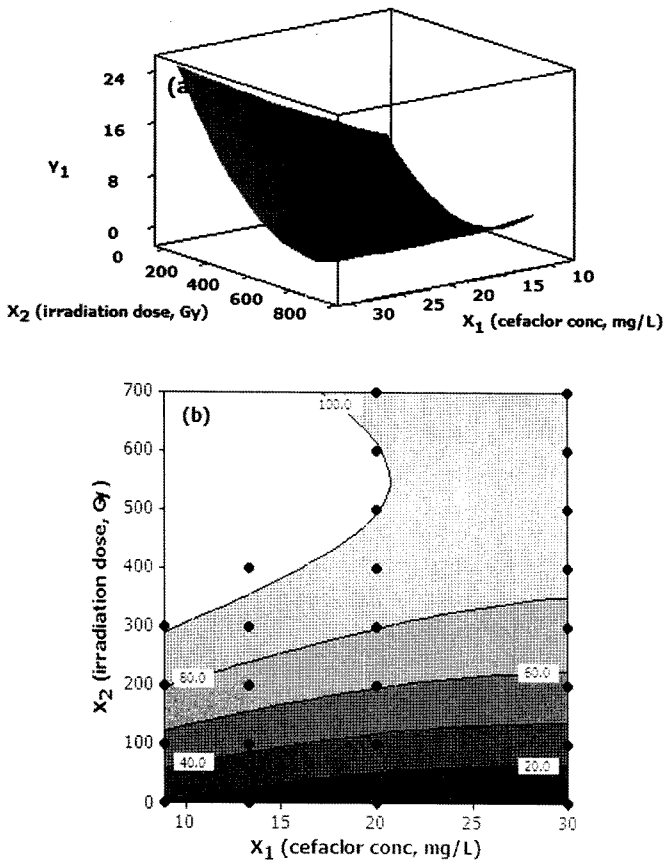


Fig. 3. Response surface plot (3D) (a) and contour plot (2D) (b) of Y_1 (cefaclor conc, mg/L). The response surface plots represent the effect of the significant variables (X_1, X_2) and their interaction (X_1^1, X_1^2, X_2^2) in the response variable.

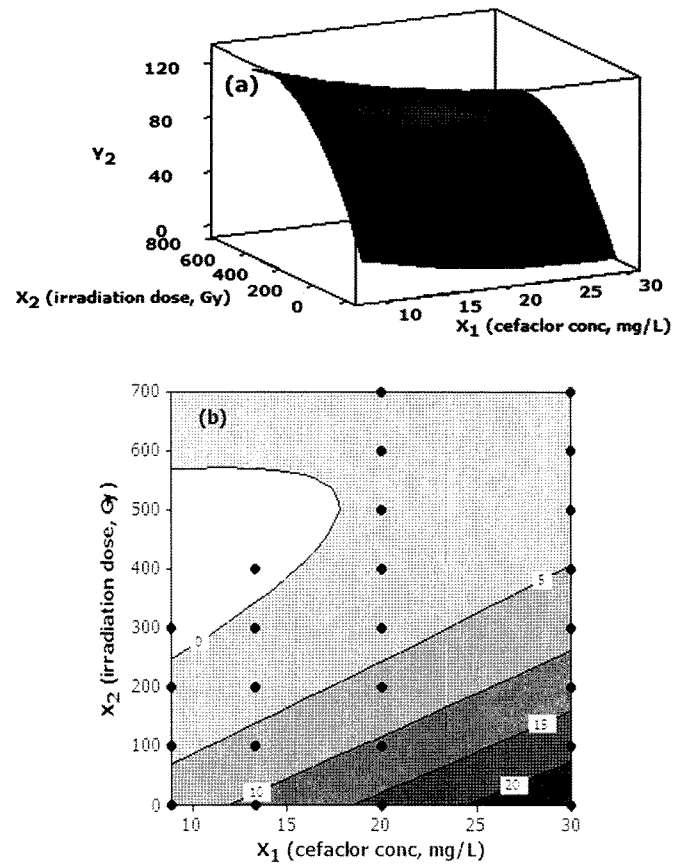


Fig. 4. Response surface plot (3D) (a) and contour plot (2D) (b) of Y_2 (removal efficiency (%). The response surface plots represent the effect of the significant variables (X_1, X_2) and their interaction (X_1^1, X_1^2, X_2^2) in the response variable.

Further, the coordinates of local minima in terms of the processing variables were determined by differentiating Eq. (14) for cefaclor concentration and Eq. (15) for gamma-ray dose in terms of X_1 and X_2, and setting the result obtained equal to zero, according to [10]:

$$\left[\frac{\partial t_{Cefaclor}}{\partial X_1}\right]_{X_2} = 0 \tag{14}$$

$$\left[\frac{\partial t_{gamma-ray}}{\partial X_2}\right]_{X_1} = 0 \tag{15}$$

The conditions obtained at the minimum point for Y_1 response were X_1 = 25 mg/L and X_2 = 350 Gy, while those at the maximum point for total Y_2 response were X_1 = 21 mg/L and X_2 = 560 Gy. In general, higher gamma radiation and lower cefaclor concentration favorably improved the percentage cefaclor removal. It may therefore be necessary to make an acceptable compromise in all the response results. However, it should be noted that the above general observations were only true for the regions bound by the stationary point.

3.6. Model Validation and Confirmation

Verification experiments performed at the predicted conditions derived from the ridge analysis of the response surface demonstrated that the experimental values were reasonably close to the predicted values, as shown in Table 6, thereby confirming the validity and adequacy of the predicted model. Table 6 illustrates the observed and predicted responses and residual values for the reduction of cefaclor concentration (Y_1) and cefaclor removal (%) (Y_2), performed at the optimal values of the cefaclor concentration (X_1) and gamma-ray dose (X_2) parameters in-

Table 6. Optimal conditions and comparison of observed and predicted responses

Response	Optimal conditions	Predicted value[a]	Observed value[b]	Residual
Y_1	X_1 = 25 mg/L, X_2 = 350 Gy	4.37 mg/L	3.58 mg/L	0.79
Y_2	X_1 = 21 mg/L, X_2 = 565 Gy	98.35%	98.37%	0.07

[a]Predicted value using ridge analysis of response, [b]Observed value using additional experiments (5 trials) at the optimal conditions

vestigated in this study. The results presented in Table 6 support the conclusion that an optimized combination of the investigated parameters ensured the reduction of cefaclor concentration (Y_1) and cefaclor removal (%) (Y_2), to levels which were very close to the predicted values.

4. Conclusions

The gamma irradiation degradation of cefaclor was optimized and modeled by using an experimental design. The study results demonstrated the effectiveness of a radiolytic treatment in the removal of cefaclor in an aqueous solution. The ensuring mathematical modeling could predict the gamma irradiation decomposition at any point in the experimental domain, as well as determine the optimal degradation conditions. The high correlation in the model confirmed the capability of the second-order polynomial model to optimize the gamma ray treatment of cefaclor. The conditions required to attain a Y_1 response for cefaclor reduction of 4.37 mg/L were a cefaclor concentration of 25 mg/L (X_1) and a gamma irradiation level of 350 Gy (X_2), while those for a Y_2 response for cefaclor removal of 98.35 % were a cefaclor concentration of 21 mg/L (X_1) and a gamma irradiation level of 565 Gy (X_2). Under these optimized conditions, the experimental values presented good agreement with the value predicted by ridge analysis, suggesting that such optimization based on experimental design and statistical analysis can increase the efficiency in optimum condition estimation. In conclusion, the application of radiation technology has shown promise as an effective alternative for the treatment of industrial and municipal effluents containing various antibiotic compounds. This study also demonstrated that the response surface methodology is a valuable tool to allow engineers to determine the optimal operating conditions for maximizing cefaclor removal by radiation technology.

References

1. Calza, P., Sakkas, V. A., Medana, C., Baiocchi, C., Dimou, A., Pelizzetti, E., and Albanis, T., "Photocatalytic degradation study of diclofenac over aqueous TiO_2 suspensions," *Applied Catalysis. B: Environment*, **67**, 197-205 (2006).
2. Carballa, M., Omil, F., Lema, J. M., Llompart, M., García-Jares, C., Rodríguez, I., Gómez, M., and Ternes, T., "Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant," *Water Res.*, **38**(12), 2918-2926 (2004).
3. Costanzo, S. D., Murby, J., and Bates, J., "Ecosystem response to antibiotics entering the aquatic environment," *Mar. Pollut. Bull.*, **51**(1-4), 218-223 (2005).
4. Batt, A. L., Bruce, I. B., and Aga, D. S., "Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges," *Environ. Pollut.*, **142**(2), 295-302 (2006).
5. Jørgensen, S. E. and Halling-Sørensen, B., "Drugs in the environment," *Chemosphere*, **40**(7), 691-699 (2000).
6. Göbel, A., McArdell, C. S., Suter, M. J.-F., and Giger, W., "Trace determination of macrolide and sulfonamide antimicrobials, a human sulfonamide metabolite, and trimethoprim in wastewater using liquid chromatography coupled to electrospray tandem mass spectrometry," *Anal. Chem.*, **76**(16), 4756-4764 (2004).
7. Díaz-Cruz, M. S., López de Alda, M. J., and Barceló, D., "Environmental behavior and analysis of veterinary and human drugs in soils, sediments and sludge," *Trends in Analytical Chemistry*, **22**(6), 340-351 (2003).
8. Heberer, T., "Tracking persistent pharmaceutical residues from municipal sewage to drinking water," *J. Hydrol.*, **266**(3-4), 175-189 (2002).
9. Hirsch, R., Ternes, T., Haberer, K., and Kratz, K. L., "Occurrence of antibiotics in the aquatic environment," *Sci. of the Total Environment*, **225**, 109-118 (1999).
10. Cho, I. H. and Zoh, K. D., "Photocatalytic Degradation of azo dye (Reactive Red 120) in TiO_2/UV System: Optimization and Modeling using a Response Surface Methodology (RSM) based on the Central Composite Design," *Dye and Pigments*, **75**(3), 533-543 (2007).
11. Elibol, M., "Optimization of medium composition for actinorhodin production by Streptomyces coelicolor A3(2) with response surface methodology," *Proc. Biochem.*, **39**, 1057-1062 (2004).
12. Olivera, F. C., Caron, G. R., and Brandelli, A., "Bacteriocin production by Bacillus licheniformis strain P40 in cheese whey using response surface methodology," *Biochem. Eng. J.*, **21**, 53-58 (2004).
13. Mamani, M. C. V., Farfan, J. A., Reyes, F. G. R. R., and Rath, S., "Simultaneous determination of tetracyclines in pharmaceuticals by CZE using experimental design," *Talanta*, **70**, 236-243 (2006).
14. Joshi, S., "HPLC separation of antibiotics present in formulated and unformulated samples," *J. Pharm. Biomed. Anal.*, **28**, 795-809 (2002).
15. Khan, S. J. and Ongerth, J. E., "Estimation of pharmaceutical residues in primary and secondary sewage sludge based on quantities of use and fugacity modeling," *Water Sci. Technol.*, **46**(3), 105-113 (2002).
16. Park, J., An approach for developing aquatic environmental risk assessment framework for pharmaceuticals in Korea, A report from Korea Environment Institute, KEI-RE-05.
17. Bielski, B. H. J., Cabelli, D. E., and Arudi, R. L., "Reactivity of $HO_2\surd/O_2\surd-$ radicals in aqueous solution," *J. Phys. Chem.*, **14**(4), 1041-1100 (1985).
18. Buxton, G. V., Langan, J. R., and Smith, J. R. L., "A radiation chemical study of the oxidation of hydroxycyclohexadienyl radicals, *J. Phys. Chem.*, **90**, 6309-6313 (1986).
19. Zona, R. and Solar, S., "Oxidation of 2,4-dichlorophenoxyacetic acid by ionizing radiation: degradation, detoxification and mineralization," *Radiat. Phys. Chem.*, **66**(2), 137-143 (2003).
20. Box, G. and Hunter, W. G., Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building, Wiley, NJ (1987).