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Robust Nonlinear Least Squares Approaches for Evaluating OVA-Mediated Bleaching Reactions: An Experimental Comparative Study

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Abstract

Nonlinear models are usually encountered in various areas including experimental studies such as physics, chemistry, biology etc. Ordinary least squares is one of the most widely used methods for parameter estimation in different types of nonlinear models. However, there are some regression assumptions need to be satisfied for obtaining efficient parameter estimates. In this paper, the parameter estimation process is evaluated carefully for some bleaching reactions by using chicken egg albumin (OVA) and some precautions are taken in the presence of violations of the assumptions (heteroscedasticity, autocorrelation, the presence of outliers). In this way, robust logged nonlinear least squares approaches are examined and compared under different conditions of reactions. It can be concluded that logged and robust analyses are preferable together in nonlinear regression in order to obtain efficient parameter estimates and reliable results. However, the best weight function in robust nonlinear least squares can vary for each condition.

1. INTRODUCTION

Nonlinear regression analysis is an inevitable process for most researchers examining the relationship among several variables obtained from scientific experimental studies. In recent years, the use of nonlinear models has been a requirement in most scientific areas. Therefore, the analysis of nonlinear models should be performed carefully in order to obtain efficient results. One of the most widely used methods is known as ordinary least squares (OLS) for nonlinear parameter estimation. Over many years, OLS based approaches have been used for different aims in many scientific areas dealing with nonlinear models [1-9]. Some assumptions should be satisfied in order to obtain efficient parameter estimates by using OLS in linear and nonlinear models. In literature, these assumptions are given in detail and some approaches are proposed in the presence of violations of them.

Variance-stabilizing transformations and robust estimation methods are used in the presence of heteroscedastic errors in linear models [10,11]. Some new approaches are also proposed to detect and correct different types of heteroscedasticity in linear regression [12,13]. Moreover, the problem of heteroscedasticity is examined in nonlinear models and some modified methods are given [14,15]. The problem of autocorrelated errors is also evaluated in linear and nonlinear regression models basically [16-18]. Some modified approaches related with two-stage least squares method are examined in nonlinear regression in the presence of errors derived from different wide-sense stationary autoregressive models [19,20]. One another problem is that OLS analysis can be totally disrupted in the presence of outliers. Therefore, such observations should be examined in each model carefully and the use of *M*/*S*-estimators can have a high importance to obtain robust parameter estimates [21]. Some modified robust estimators in nonlinear regression with autocorrelated errors are also investigated [22,23].

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In some areas, the violations of assumptions can be ignored in the analysis stage. Thus, the results of the analysis can have a breakdown. Especially, nonlinear models including complexity are required attention for parameter estimation to achieve consistent results. One type of these nonlinear models known as compartmental models is usually encountered in physical or chemical reactions. They are defined as a system which consists of a finite number of macroscopic subsystems [24].

In this paper, an experimental data related with bleaching reactions under different pH level and different chicken egg albumin (OVA) concentration is fitted by a compartmental model with four compartments. The assumptions are examined after OLS and some approaches are used as alternative to OLS in order to hinder the violations of assumptions. In this way, robust nonlinear least squares method with different robust weight functions is investigated. The selection of the best and plausible one is discussed with the comparison of efficient parameter estimation. The rest of this paper is organized as follows. The data and model description, the theoretical overview of OLS and the robust approaches are given in section 2. The results are presented in section 3. The conclusion and discussion are provided in section 4.

2. MATERIALS AND METHODS

2.1. A General View of Nonlinear Least Squares Analysis

A nonlinear regression model can be defined by

$$y_i = f(\boldsymbol{x_i}, \boldsymbol{\theta}) + \varepsilon_i, \quad i = 1,\ 2,\ \ldots,\ n \tag{1}$$

where $\boldsymbol{\theta}$ is a $p\times 1$ vector of unknown parameters and ε_i is the random error term satisfying assumptions. The least squares function is

$$S(\boldsymbol{\theta}) = \sum_{i=1}^{n}\left(y_i - f\left(\boldsymbol{x_i}, \boldsymbol{\theta}\right)\right)^2 \tag{2}$$

and the normal equations

$$\sum_{i=1}^{n}\left(y_i - f\left(\boldsymbol{x_i}, \boldsymbol{\theta}\right)\right)\frac{\partial f\left(\boldsymbol{x_i}, \boldsymbol{\theta}\right)}{\partial \theta_j}\Bigg|_{\boldsymbol{\theta}=\hat{\boldsymbol{\theta}}} = 0, \quad j = 1,\ 2,\ \ldots,\ p \tag{3}$$

should be solved in order to obtain the OLS estimate $\hat{\boldsymbol{\theta}}$. Because of the difficulty of solving them some numerical methods can be used. Equation (3) can be given in matrix form

$$\boldsymbol{F}'(\boldsymbol{\theta})\left[\boldsymbol{Y} - \boldsymbol{f}(\boldsymbol{\theta})\right]\Big|_{\boldsymbol{\theta}=\hat{\boldsymbol{\theta}}} = \boldsymbol{0} \tag{4}$$

where $\boldsymbol{F}(\boldsymbol{\theta}) = \partial \boldsymbol{f}(\boldsymbol{\theta})/\partial \boldsymbol{\theta}'$. By using the linear Taylor approximation about a vector of initial values $\boldsymbol{\theta}^0$,

$$\boldsymbol{f}\left(\boldsymbol{\theta}\right) \approx \boldsymbol{f}\left(\boldsymbol{\theta}^0\right) + \frac{\partial \boldsymbol{f}\left(\boldsymbol{\theta}^0\right)}{\partial \boldsymbol{\theta}'}\left(\boldsymbol{\theta} - \boldsymbol{\theta}^0\right) \tag{5}$$

and considering equation (4) again,

$$\boldsymbol{\theta} - \boldsymbol{\theta}^0 = \left[\boldsymbol{F}'\left(\boldsymbol{\theta}^0\right)\boldsymbol{F}\left(\boldsymbol{\theta}^0\right)\right]^{-1}\boldsymbol{F}'\left(\boldsymbol{\theta}^0\right)\left[\boldsymbol{Y} - \boldsymbol{f}\left(\boldsymbol{\theta}^0\right)\right] = \boldsymbol{\delta}^0 \tag{6}$$

is obtained. Here, $\boldsymbol{\theta}^1 = \boldsymbol{\theta}^0 + \boldsymbol{\delta}^0$ and it provides an iterative scheme for determining the OLS estimate $\hat{\boldsymbol{\theta}}$. Equation (6) is known as the Gauss-Newton method [25].

2.2. Robust Nonlinear Least Squares Analysis

If there are outliers in the dataset, a robust estimation method can be necessary. Instead of minimizing the sum of squared residuals, an appropriate loss function

$$\boldsymbol{h}(\boldsymbol{\theta}) = \sum_{i=1}^{n} \rho\left(\frac{y_i - f(\boldsymbol{x_i}, \boldsymbol{\theta})}{\sigma}\right) \tag{7}$$

is minimized in order to find a robust estimator. A class of robust estimators is known as *M*-estimators. The *M*-estimate $\tilde{\boldsymbol{\theta}}$ is the solution of

$$\sum_{i=1}^{n} \psi\left(\frac{y_i - f(\boldsymbol{x_i}, \boldsymbol{\theta})}{\tilde{\sigma}}\right) \left.\frac{\partial f(\boldsymbol{x_i}, \boldsymbol{\theta})}{\partial \theta_j}\right|_{\boldsymbol{\theta}=\tilde{\boldsymbol{\theta}}} = 0, \quad j = 1,\ 2,\ \ldots,\ p \tag{8}$$

where $\psi = \rho'$ is an influence function and $\tilde{\sigma}$ is a robust estimate of dispersion [25]. The weight function is defined by

$$w(z_i) = \psi(z_i)/z_i \tag{9}$$

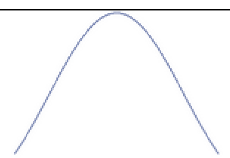
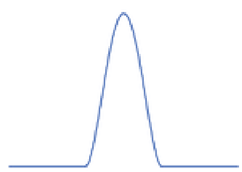
where $z_i = \left(y_i - f(\boldsymbol{x_i}, \boldsymbol{\theta})\right)/\tilde{\sigma}$. By using the positive diagonal matrix of weights $\boldsymbol{W}$, equation (6) can be rewritten as

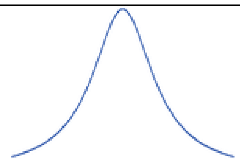
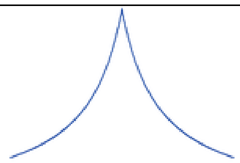
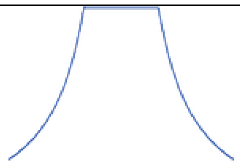
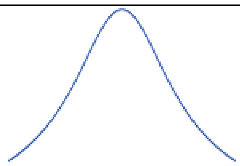
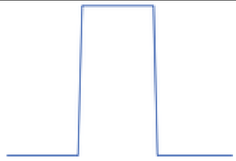
$$\boldsymbol{\theta} - \boldsymbol{\theta}^0 = \left[\boldsymbol{F}'(\boldsymbol{\theta}^0)\boldsymbol{W}^0\boldsymbol{F}(\boldsymbol{\theta}^0)\right]^{-1} \boldsymbol{F}'(\boldsymbol{\theta}^0)\boldsymbol{W}^0\left[\boldsymbol{Y} - \boldsymbol{f}(\boldsymbol{\theta}^0)\right] = \boldsymbol{\delta}^0 \tag{10}$$

This modified Gauss-Newton method becomes an iteratively reweighted least squares algorithm [25].

Some robust loss functions and the corresponding weight functions are given in Table 1 [26].

Table 1. *Some common robust functions*

Name	Loss Function $\rho(z_i)$	Weight Function $w(z_i)$	Weight Graph
Andrews	$\begin{cases} 1-\cos(z_i/1.339) & \text{if } \lvert z_i\rvert \le 1.339\pi \\ 2 & \text{if } \lvert z_i\rvert > 1.339\pi \end{cases}$	$\begin{cases} \dfrac{\sin(z_i/1.339)}{z_i/1.339} & \text{if } \lvert z_i\rvert \le 1.339\pi \\ 0 & \text{if } \lvert z_i\rvert > 1.339\pi \end{cases}$	
Bisquare	$\begin{cases} \dfrac{4.685^2}{6}\left(1-\left[1-\left(\dfrac{z_i}{4.685}\right)^2\right]^3\right) & \text{if } \lvert z_i\rvert \le 4.685 \\ \dfrac{4.685^2}{6} & \text{if } \lvert z_i\rvert > 4.685 \end{cases}$	$\begin{cases} \left[1-\left(\dfrac{z_i}{4.685}\right)^2\right]^2 & \text{if } \lvert z_i\rvert \le 4.685 \\ 0 & \text{if } \lvert z_i\rvert > 4.685 \end{cases}$	

Cauchy	$\dfrac{2.385^2}{2}\ln\left[1+\left(\dfrac{z_i}{2.385}\right)^2\right]$	$\dfrac{1}{1+\left(\dfrac{z_i}{2.385}\right)^2}$	
Fair	$1.4^2\left[\dfrac{\lvert z_i\rvert}{1.4}-\ln\left(1+\dfrac{\lvert z_i\rvert}{1.4}\right)\right]$	$\dfrac{1}{1+\dfrac{\lvert z_i\rvert}{1.4}}$	
Huber	$\begin{cases} z_i^2/2 & \text{if } \lvert z_i\rvert \le 1.345 \\ 1.345\lvert z_i\rvert-\dfrac{1.345^2}{2} & \text{if } \lvert z_i\rvert > 1.345 \end{cases}$	$\begin{cases} 1 & \text{if } \lvert z_i\rvert \le 1.345 \\ \dfrac{1.345}{\lvert z_i\rvert} & \text{if } \lvert z_i\rvert > 1.345 \end{cases}$	
Logistic	$1.205^2\ln\left(\cosh\left(z_i/1.205\right)\right)$	$\dfrac{\tanh\left(z_i/1.205\right)}{z_i/1.205}$	
Talwar	$\begin{cases} z_i^2/2 & \text{if } \lvert z_i\rvert \le 2.795 \\ 2.795^2/2 & \text{if } \lvert z_i\rvert > 2.795 \end{cases}$	$\begin{cases} 1 & \text{if } \lvert z_i\rvert \le 2.795 \\ 0 & \text{if } \lvert z_i\rvert > 2.795 \end{cases}$	

2.3. Data and Model Description

Chicken egg albumin called ovalbumin (OVA) is the major protein found in egg white. It is a non-inhibitory member of the serpin superfamily [27]. The OVA effect is requested to be investigated on the nonphotochemical bleaching of malachite green in aqueous solution and therefore, a part of data used in [8] is tried to be evaluated. OVA and other related materials are provided from Sigma Chemical Co., USA [8]. The reactions are carried out under different and adequate conditions in order to observe the bleaching process. In this paper, there are nine different experimental datasets about the bleaching process according to time (min) which are tried to be fitted on a nonlinear compartmental model. Two different conditions are handled in these bleaching reactions. The first one is the pH level and the second one is the OVA concentration. The bleaching reactions occur at two different pH levels; 10 and 8. Moreover, the reactions include six different OVA concentrations; 100mL, 200mL, 400mL, 800mL, 1500mL and 3000mL. The scatter plots concerned with the bleaching reactions obtained under two different pH levels are given in Figure 1.

It is proposed that the bleaching process can be analyzed by a compartmental model including four compartments given by

$$y_i=\alpha_1\exp(-\beta_1 t_i)+\alpha_2\exp(-\beta_2 t_i)+\alpha_3\exp(-\beta_3 t_i)+\alpha_4\exp(-\beta_4 t_i)+\varepsilon_i,\qquad i=1,\ 2,\ \ldots,\ n \tag{11}$$

where ε_i should be identically independently distributed with zero mean and constant variance. Equation (11) shows the relationship between milli absorbance (mA) units and time (min).

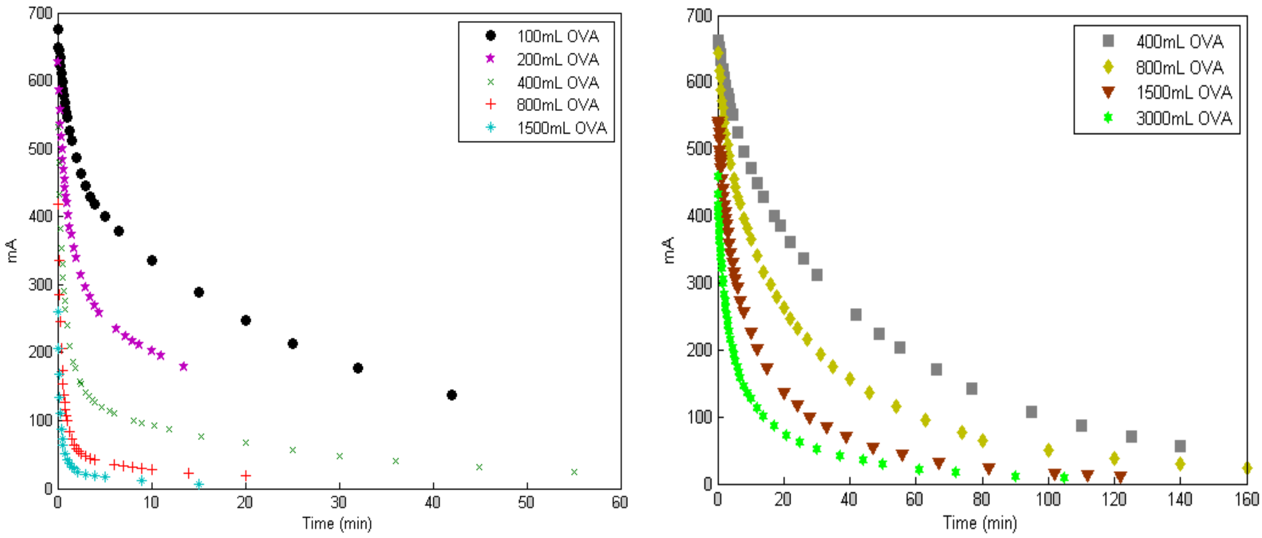


Figure 1. *Scatter plot of different bleaching reactions at; a) pH 10 b) pH 8*

3. RESULTS

The aim of this study is to make comparison of several nonlinear approaches on a real, highly nonlinear and difficult for depth analyzable datasets. Therefore, the datasets arranged under different conditions are examined in view of non-robust and robust approaches by using MATLAB R2015b. Firstly, OLS is used for parameter estimation and the results obtained from this analysis are given in Table 2. According to Table 2, except for some parameter estimates the others seem significant. However, standart errors (SE) and the other statistics obtained from OLS are doubtful because of the violations of assumptions shown in

Table 2. *The results of OLS analysis*

Bleaching pH	OVA concentration	$\hat{\alpha}_1$ $\hat{\alpha}_2$ $\hat{\alpha}_3$ $\hat{\alpha}_4$	SE	t	p-value	$\hat{\beta}_1$ $\hat{\beta}_2$ $\hat{\beta}_3$ $\hat{\beta}_4$	SE	t	p-value
10	100mL	36.473	20.409	1.787	0.089	2.717	1.375	1.976	0.062
		187.792	15.624	12.020	1.3e-10**	0.634	0.097	6.517	2.4e-06**
		454.457	21.213	21.423	2.9e-15**	0.032	0.006	5.154	4.8e-05**
		4.335	29.852	0.145	0.886	-0.036	0.121	-0.296	0.770
	200mL	121.655	14.436	8.427	3.5e-08**	10.014	2.301	4.352	2.8e-04**
		133.812	31.354	4.268	3.4e-04**	1.685	0.443	3.806	0.001**
		218.044	36.001	6.057	5.2e-06**	0.545	0.076	7.151	4.7e-07**
		278.424	6.703	41.539	1.2e-21**	0.033	0.002	15.644	4.8e-13**
	400mL	159.154	36.616	4.347	1.9e-04**	16.917	5.595	3.023	0.006**
		274.830	35.834	7.669	3.9e-08**	1.723	0.275	6.257	1.3e-06**
		115.392	42.024	2.746	0.011*	0.496	0.138	3.589	0.001**
		125.699	4.651	27.027	1.5e-20**	0.031	0.002	17.274	9.1e-16**
	800mL	226.132	12.007	18.833	3.4e-14**	10.933	1.230	8.886	2.2e-08**
		298.323	11.464	26.022	6.7e-17**	2.710	0.223	12.151	1.1e-10**
		75.069	15.150	4.955	7.6e-05**	0.742	0.113	6.555	2.2e-06**
		45.241	1.893	23.904	3.5e-16**	0.048	0.004	12.311	8.6e-11**
	1500mL	6395.695	22559.98	0.283	0.782	69.227	45.302	1.528	0.155
		247.615	10.964	22.584	1.4e-10**	6.652	0.588	11.306	2.1e-07**
		79.773	10.934	7.296	1.6e-05**	1.532	0.191	8.016	6.4e-06**
		26.954	1.754	15.365	8.8e-09**	0.091	0.011	8.402	4.1e-06**
8	400mL	206.020	425.672	0.484	0.633	0.011	0.008	1.346	0.191
		39.876	26.527	1.503	0.146	0.389	0.138	2.824	0.009**
		101.357	20.652	4.908	5.9e-05**	0.118	0.051	2.331	0.029*
		320.691	390.469	0.821	0.420	0.023	0.015	1.585	0.127

	800mL	72.030	5.843	12.328	1.7e-13**	0.997	0.115	8.649	9.1e-10**
		157.805	15.980	9.875	4.3e-11**	0.153	0.019	7.989	5.1e-09**
		348.955	23.847	14.633	1.8e-15**	0.033	0.004	7.754	9.5e-09**
		88.569	39.820	2.224	0.034*	0.009	0.003	3.314	0.002**
	1500mL	81.474	18.074	4.508	7.8e-05**	0.857	0.155	5.541	3.7e-06**
		192.454	71.783	2.681	0.011*	0.172	0.058	2.976	0.005**
		216.280	37.117	5.827	1.6e-06**	0.051	0.027	1.926	0.063
		72.682	80.362	0.904	0.372	0.016	0.009	1.752	0.089
	3000mL	111.289	27.500	4.047	2.5e-04**	27.955	5.267	5.308	5.1e-06**
		88.253	6.067	14.547	4.1e-17**	1.000	0.083	12.048	1.5e-14**
		222.061	5.033	44.120	3.0e-34**	0.197	0.009	21.809	4.3e-23**
		127.866	4.406	29.022	1.5e-27**	0.029	0.001	30.704	2.0e-28**

*: significance at $\alpha = 0.05$; **: significance at $\alpha = 0.01$

Table 3. After OLS, the residuals are examined via some graphs and statistical tests in order to check the assumptions. The results are not given here, but the satisfied assumptions are marked as "-" in Table 3. There are a lot of violations of assumptions in the OLS analysis. Therefore, logged OLS is preferred to hinder the violations or at least to decrease the effects of them. The logged OLS analysis includes taking logarithm both y_i and $f(t_i, \boldsymbol{\alpha}, \boldsymbol{\beta})$ in equation (11), which is usually preferred for compartmental models. Logged OLS is applied and the results obtained from this analysis are given in Table 4.

Table 3. *The examination of assumptions after some primary analyzes*

		OLS			Logged OLS		
Bleaching pH	OVA concentration	Heteroscedasticity	Autocorrelation	Outlier	Heteroscedasticity	Autocorrelation	Outlier
10	100mL	+	+	+	-	-	+
	200mL	+	-	+	+	-	+
	400mL	-	+	+	-	-	+
	800mL	+	-	+	-	-	+
	1500mL	+	-	+	-	-	-
8	400mL	+	-	+	-	-	+
	800mL	-	+	+	-	-	+
	1500mL	+	-	+	-	-	+
	3000mL	-	+	-	-	-	+

-: nonexisting; +: existing

Table 4. *The results of logged OLS analysis*

Bleaching pH	OVA concentration	$\hat{\alpha}_1$ $\hat{\alpha}_2$ $\hat{\alpha}_3$ $\hat{\alpha}_4$	SE	*t*	*p*-value	$\hat{\beta}_1$ $\hat{\beta}_2$ $\hat{\beta}_3$ $\hat{\beta}_4$	SE	*t*	*p*-value
10	100mL	31.332	13.859	2.261	0.035*	3.054	1.536	1.988	0.061
		189.670	11.583	16.375	4.7e-13**	0.663	0.067	9.880	3.9e-09**
		433.522	75.737	5.724	1.3e-05**	0.035	0.007	4.830	1.0e-04**
		28.791	81.461	0.353	0.727	-0.007	0.038	-0.176	0.862
	200mL	124.329	22.026	5.645	1.3e-05**	10.872	2.707	4.017	6.2e-04**
		127.500	13.976	9.123	9.4e-09**	1.834	0.328	5.594	1.5e-05**
		227.922	19.173	11.888	8.7e-11**	0.564	0.036	15.464	6.0e-13**
		279.624	2.751	101.661	9.1e-30**	0.033	0.001	39.351	3.7e-21**
	400mL	189.323	25.570	7.404	7.3e-08**	3.365	0.566	5.942	2.9e-06**
		239.294	25.635	9.335	8.7e-10**	0.891	0.089	9.991	2.2e-10**
		38.308	7.086	5.406	1.2e-05**	0.105	0.037	2.800	9.5e-03**
		104.779	10.767	9.732	3.7e-10**	0.027	0.002	14.840	3.3e-14**
	800mL	264.411	12.896	20.503	6.7e-15**	7.144	0.590	12.106	1.2e-10**
		254.712	13.023	19.558	1.7e-14**	1.992	0.105	19.007	2.8e-14**
		38.064	4.165	9.139	1.4e-08**	0.432	0.049	8.742	2.9e-08**
		41.494	0.961	43.192	3.2e-21**	0.042	0.001	30.916	2.3e-18**

	1500mL	547.813 231.506 67.674 26.324	608.707 17.326 6.713 0.385	0.900 13.362 10.081 68.396	0.387 3.8e-08** 6.8e-07** 8.1e-16**	34.770 5.829 1.373 0.088	16.000 0.487 0.085 0.002	2.173 11.977 16.201 58.524	0.052 1.2e-07** 5.1e-09** 4.5e-15**
8	400mL	76.926 50.332 104.891 435.616	116.403 16.395 10.034 98.940	0.661 3.070 10.454 4.403	0.515 0.005** 3.3e-10** 2.1e-04**	0.007 0.343 0.100 0.019	0.006 0.070 0.023 0.003	1.312 4.874 4.374 5.878	0.203 6.4e-05** 2.2e-04** 5.4e-06**
	800mL	65.359 145.842 342.552 114.952	18.008 16.278 10.240 18.528	3.629 8.959 33.453 6.204	0.001** 4.1e-10** 6.8e-26** 6.9e-07**	1.132 0.178 0.037 0.010	0.576 0.040 0.003 0.001	1.966 4.477 12.373 11.203	0.058 9.6e-05** 1.6e-13** 2.0e-12**
	1500mL	72.271 174.860 229.163 87.314	18.789 19.114 19.449 10.451	3.846 9.148 11.783 8.355	0.001** 1.4e-10** 2.3e-13** 1.2e-09**	0.950 0.198 0.058 0.017	0.287 0.039 0.006 0.001	3.308 5.038 9.787 18.095	0.002** 1.7e-05** 2.8e-11** 1.1e-18**
	3000mL	65.272 200.850 119.097 72.216	6.563 7.763 6.704 5.370	9.945 25.874 17.764 13.448	4.0e-12** 9.8e-26** 5.3e-20** 5.0e-16**	2.314 0.299 0.072 0.021	0.433 0.021 0.007 0.001	5.343 14.376 11.002 28.521	4.5e-06** 6.0e-17** 2.3e-13** 2.9e-27**

*: significance at $\alpha = 0.05$; **: significance at $\alpha = 0.01$

It can be said in view of Table 3 that the assumptions of homoscedastic and uncorrelated errors are satisfied with logged OLS analysis. Only there is a heteroscedasticity at the condition of 200mL OVA concentration and ph 10, but its effect decreases too much in comparison with this one after OLS. Therefore, the results given in Table 4 are more reliable that it can be also verified by the results given in Table 5. It is clear from Table 5 that for both of OLS and logged OLS the models are significant. However, root mean squared error (RMSE) values show that the logged OLS fits to data better than OLS for all pH and OVA concentration combinations. On the other hand, there is a problem of outliers for both OLS and logged OLS.

Table 5. *The comparison of OLS and logged OLS*

			OLS			Logged OLS		
Bleaching pH	OVA concentration	Sample size (n)	RMSE	F	p-value	RMSE	F	p-value
10	100mL	28	2.48	1.4e+05	8.6e-46**	4.3e-03	7.0e+06	9.6e-63**
	200mL	29	1.23	3.7e+05	3.0e-52**	2.6e-03	1.9e+07	2.9e-70**
	400mL	34	3.29	2.1e+04	2.0e-47**	13.0e-03	6.3e+05	9.6e-67**
	800mL	28	0.85	1.1e+05	1.2e-44**	6.9e-03	1.4e+06	7.1e-56**
	1500mL	19	0.96	2.6e+04	1.9e-22**	12.2e-03	2.5e+05	8.3e-28**
8	400mL	31	0.96	8.8e+05	3.2e-61**	2.0e-03	3.5e+07	1.4e-79**
	800mL	39	1.39	3.7e+05	5.9e-75**	12.0e-03	1.1e+06	5.4e-82**
	1500mL	41	2.09	1.4e+05	1.6e-72**	10.7e-03	1.3e+06	1.5e-88**
	3000mL	46	1.38	2.0e+05	2.6e-85**	11.2e-03	1.2e+06	3.0e-100**

**: significance at $\alpha = 0.01$

Robust nonlinear least squares method with different weight functions is used in order to obtain efficient parameter estimates and robust SE values. In this study, robust logged analysis is performed with different weight functions shown in Table 1. Approximate RMSE values obtained from this robust logged analysis are given in Table 6. It can be seen that the values are very close because the logged analysis reduces RMSE values seriously. The approach marked as bold in Table 6 provides the most efficient, robust and plausible (not completely removed the effects but less affected from outliers; also taking into account some preliminary information) parameter estimates for each condition in this experimental study. It can be emphasized that the best approach differs for each condition. Moreover, it can be said that nearly same parameter estimates are obtained in some cases by using Andrews and bisquare weight functions. The results obtained from robust logged analysis with the best approach are given in Table 7.

Table 6. *The comparison of different weight functions*

Bleaching pH	OVA concentration	Sample size (n)	RMSE (Andrews)	RMSE (Bisquare)	RMSE (Cauchy)	RMSE (Fair)	RMSE (Huber)	RMSE (Logistic)	RMSE (Talwar)
10	100mL	28	3.9e-03	3.9e-03	4.1e-03	4.3e-03	4.0e-03	4.1e-03	**3.9e-03**
	200mL	29	2.4e-03	2.4e-03	2.4e-03	2.6e-03	**2.4e-03**	2.5e-03	2.5e-03
	400mL	34	**10.7e-03**	**10.7e-03**	10.7e-03	11.3e-03	10.9e-03	11.0e-03	10.8e-03
	800mL	28	7.3e-03	7.3e-03	7.5e-03	8.7e-03	**6.9e-03**	7.7e-03	6.9e-03
	1500mL	19	13.9e-03	13.9e-03	13.9e-03	16.8e-03	**12.2e-03**	14.3e-03	13.0e-03
8	400mL	31	1.8e-03	1.8e-03	1.9e-03	1.9e-03	1.8e-03	1.9e-03	**1.8e-03**
	800mL	39	**9.6e-03**	**9.6e-03**	9.8e-03	9.8e-03	9.7e-03	9.8e-03	9.7e-03
	1500mL	41	**8.6e-03**	**8.6e-03**	8.6e-03	9.0e-03	8.7e-03	8.8e-03	8.6e-03
	3000mL	46	8.9e-03	8.9e-03	8.9e-03	9.1e-03	9.0e-03	9.0e-03	**8.9e-03**

It can be pointed out in view of Table 7 that some parameter estimates become significant because of robust SE. Moreover, almost all parameter estimates have a slight or more difference compared with the others obtained by using OLS or logged OLS. All the results achieved under the violations of assumptions point to the importance of robust approaches especially for highly nonlinear models.

Table 7. *The results of robust logged analysis*

Bleaching pH	OVA concentration	$\hat{\alpha}_1$ $\hat{\alpha}_2$ $\hat{\alpha}_3$ $\hat{\alpha}_4$	SE	t	p-value	$\hat{\beta}_1$ $\hat{\beta}_2$ $\hat{\beta}_3$ $\hat{\beta}_4$	SE	t	p-value
10	100mL	38.096 188.769 436.398 25.478	11.103 9.205 60.816 65.925	3.431 20.508 7.176 0.386	0.003** 6.7e-15** 6.0e-07** 0.703	3.219 0.657 0.035 -0.008	1.153 0.057 0.006 0.035	2.792 11.439 5.653 -0.234	0.011* 3.2e-10** 1.6e-05** 0.817
	200mL	126.380 128.362 233.854 280.049	31.546 10.596 14.613 2.406	4.006 12.114 16.003 116.383	6.4e-04** 6.1e-11** 3.1e-13** 5.4e-31**	12.334 1.980 0.573 0.033	3.408 0.297 0.030 0.001	3.619 6.670 19.335 44.447	0.002** 1.3e-06** 7.4e-15** 2.9e-22**
	400mL	190.574 226.204 36.943 116.881	34.865 30.914 9.433 2.700	5.466 7.317 3.916 43.292	9.9e-06** 9.0e-08** 0.001** 9.2e-26**	3.124 0.941 0.192 0.029	0.536 0.132 0.052 0.001	5.829 7.109 3.686 53.134	3.8e-06** 1.5e-07** 0.001** 4.7e-28**
	800mL	266.767 254.010 37.827 41.459	12.663 12.699 4.098 0.965	21.067 20.002 9.230 42.944	4.0e-15** 1.1e-14** 1.2e-08** 3.6e-21**	7.165 1.986 0.430 0.042	0.580 0.103 0.049 0.001	12.364 19.285 8.742 30.783	8.0e-11** 2.2e-14** 2.9e-08** 2.5e-18**
	1500mL	547.838 231.506 67.674 26.324	608.883 17.325 6.713 0.385	0.900 13.363 10.081 68.397	0.388 3.8e-08** 6.8e-07** 8.1e-16**	34.770 5.829 1.373 0.088	16.002 0.487 0.085 0.002	2.173 11.978 16.202 58.524	0.053 1.2e-07** 5.1e-09** 4.5e-15**
8	400mL	73.134 49.778 106.492 438.841	100.533 12.742 8.828 85.321	0.727 3.907 12.062 5.143	0.474 0.001** 2.0e-11** 3.3e-05**	0.007 0.365 0.099 0.019	0.005 0.064 0.019 0.003	1.400 5.726 5.268 6.753	0.175 7.9e-06** 2.4e-05** 6.9e-07**
	800mL	79.199 146.013 298.342 145.747	14.768 18.505 12.810 20.500	5.363 7.891 23.291 7.110	7.6e-06** 6.6e-09** 3.3e-21** 5.5e-08**	0.929 0.148 0.038 0.012	0.290 0.033 0.004 0.001	3.207 4.474 9.518 15.001	0.003** 9.6e-05** 1.0e-10** 9.3e-16**
	1500mL	74.996 172.197 223.082 100.246	12.004 16.918 17.440 7.808	6.247 10.178 12.791 12.838	4.7e-07** 1.0e-11** 2.4e-14** 2.2e-14**	1.120 0.204 0.062 0.018	0.247 0.031 0.005 0.001	4.545 6.486 11.887 29.064	7.0e-05** 2.3e-07** 1.8e-13** 4.3e-25**
	3000mL	47.397 170.060 135.782 93.534	8.101 8.578 10.655 2.306	5.851 19.826 12.744 40.564	9.1e-07** 1.2e-21** 2.7e-15** 6.8e-33**	2.104 0.361 0.101 0.024	0.524 0.034 0.007 0.0003	4.013 10.569 15.438 84.628	2.7e-04** 7.2e-13** 5.9e-18** 6.9e-45**

*: significance at α = 0.05; **: significance at α = 0.01

4. CONCLUSION AND DISCUSSION

Researchers often need nonlinear models to analyze the relationship among variables. At first glance, OLS comes to mind for nonlinear regression analysis. However, it requires several assumptions and these assumptions can be ignored in some areas. Therefore, the assumptions should be checked carefully and necessary precautions should be taken in order to obtain efficient results. In this paper, an original experiment and its results are handled by using a nonlinear model known as compartmental model. Because of the violations of assumptions robust logged analysis is performed with some different weight functions. The joint use of robust and logged analysis improves the efficiency and reliability for both parameter estimation and test results. On the other hand, different weight functions can be preferred under different conditions of OVA-mediated bleaching reactions. As a conclusion, it is emphasized that highly nonlinear conditions can require robust logged analysis.

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CONFLICT OF INTEREST

No conflict of interest was declared by the author.

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