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Investigation of Statistical Experimental Design Results Showing UV-Vis Absorbance Values of Characterized Zn and ZnO Nanoparticles

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Abstract
Nanoparticles (NPs), whose current potential to be studied are increasing day by day due to their important physical and chemical properties, also show toxic effects in aquatic and terrestrial ecosystems. In this context, it is important to make environmental/ecological risk assessments. The properties of NPs to be used should be determined also in the toxicity studies.
Ultraviolet-visible (UV-Vis) spectroscopy results for the characterization of Zn (40-60 nm) and Zn (80-100 nm) and ZnO (10-30 nm) NPs used in our study were examined and evaluated with statistical experimental design results. According to the results of the study, the models created for all NPs were statistically significant at 95% significance level; the order of action was found to be in the form of time and dose for Zn (40-60 nm) and Zn (80-100 nm) NPs and equal for ZnO (10-30 nm) NP. Intensive and possible misuse of nanoscale materials is one of the greatest threats to the environment and all living things. In this study, important results were obtained on the establishment of control mechanisms for uses of NP.

Keywords: Zn, ZnO, Nanoparticle, UV-Vis Absorbance Values, Statistical Experimental Design

INTRODUCTION

Nanotechnology (NT) is a new scientific horizon that has been revolutionizing human life and our perception of chemistry of materials whose sizes vary from 1-100 nm (Huber, 2005). Nowadays, the NT is one of the fastest growing industry and the nanoparticles (NPs) account for a great deal of advancement in many industry fields (Colvin, 2003). The NPs or Nanomaterials are continuously developing with their unique physical and chemical properties (Vance et al., 2015). The literature on the toxicity of NPs to aquatic organisms is also rapidly developing (Garner et al., 2015), because of big potentials to be released into aquatic and terrestrial environments (Nowack et al., 2012; Batley et al., 2013). But it is a challenge to keep pace with demands for ecological risk assessments of new products (Baysal et al., 2019). At the same time, one of the important properties of the metal oxide NPs is the sorption of other metals from the media (Altıntıg et al., 2017; Baysal and Saygın, 2018).

Characteristic properties of materials vary depending on their structure and physicochemical processes used in their formulation and manufacture. Materials in nano-scale could demonstrate entirely different properties than their bulk forms. Therefore, physical and chemical properties, such as particle size, shape, surface area, surface reactivity and solubility of NPs should be carefully identified when bio-assays to determine toxicity of NPs smaller than 100 nm are to be conducted.

Ultraviolet-visible (UV-Vis) spectroscopy is one of that identification/characterization analyses, usually used for measuring molecules or inorganic ions and complexes in the solutions. While the UV scan is corresponding normally to the wavelength range of 200 - 400 nm, the visible light scan corresponds to the range 400 - 800 nm. Metal-based NPs have optical properties sensitive to size, shape, density, aggregation and refractive index close to the surface, which is why it is important to identify, characterize and examine NPs by UV-Vis spectroscopy.

MATERIAL and METHOD

Nanoparticles

Zn (40-60 nm and 80-100 nm) and ZnO (10-30 nm) were obtained from commercial companies selling SkySpring products in our country. All chemicals of analytical reagent class are used without any purification or distillation.

Statistical Determination of UV-Vis Absorbance Values

UV – visible spectra of NPs were determined at 300-800 nm wavelengths in absorption mode using UV – Vis spectrophotometer (Optima, SP-3000 Nano). In order to determine the effect of NPs in the sizes of Zn (40-60 nm), Zn (80-100) and ZnO (10-30 nm), UV-Vis absorbance values were determined by Design Expert v.10., were evaluated statistically.

RESULTS and DISCUSION

Statistical Experimental Design Results Demonstrating UV-Vis Absorbance Values of Nanoparticles

To the data obtained from the experimental design according to Table 1 and 2, it was made the regression process with the least squares method. According to the polynomial equation obtained as a result of this three-variable and three-level design, the following equations (1,2) provided us the response surface graphs.

$$yi= \beta_0+ \Sigma \beta_i X_i + \Sigma \beta_{ii} X_{ii}^2+\Sigma\beta_{ij} X_i X_j \quad (1)$$

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (2)$$

Table 1. Level and range of independent variables used in measuring of the absorbance

Variables	Variable Codes	Levels		
		-1	0	+1
Nanoparticle dose (mgL^{-1})	X1	1	10	50
Activation time (Hour)	X2	24	36	48

Table 2. Experimental design based on BBD (Box Behnken Design) model with two independent variables

No	X1: Dose	X2: Time	Zn (40-60 nm)	Zn (80-100 nm)	ZnO (10-30 nm)
1	10.00	48.00	0.124	0.176	0.176
2	10.00	24.00	0.303	0.359	0.453
3	10.00	48.00	0.123	0.179	0.179
4	50.00	48.00	0.014	0.059	0.261
5	1.00	48.00	0.054	0.014	0
6	10.00	48.00	0.118	0.166	0.161
7	10.00	72.00	0.062	0.094	0.05
8	50.00	72.00	0	0	0.145
9	10.00	48.00	0.118	0.169	0.163
10	1.00	24.00	0.074	0.05	0.041
11	50.00	24.00	0.118	0.204	0.421
12	10.00	48.00	0.12075	0.1725	0.16975
13	1.00	72.00	0.057	0.011	0

In order to find the difference of absorbance values on the basis of second order polynomial equation; statistical analysis of NP concentration (ppm) and activation time was performed and ANOVA analysis within 95% confidence value ($P<0.05$) of the model was examined. The model was compared with the experimental findings. The statistical analysis of the mean effects of NPs on the media was uploaded to the system as tables in a multi-media system (due to the image size). In the statistical evaluation, the suitability of the selected model is shown as tables for each NP. Accordingly, the quadratic model was used for all of these experiments and the Anova Test results of the obtained data are shown in the tables.

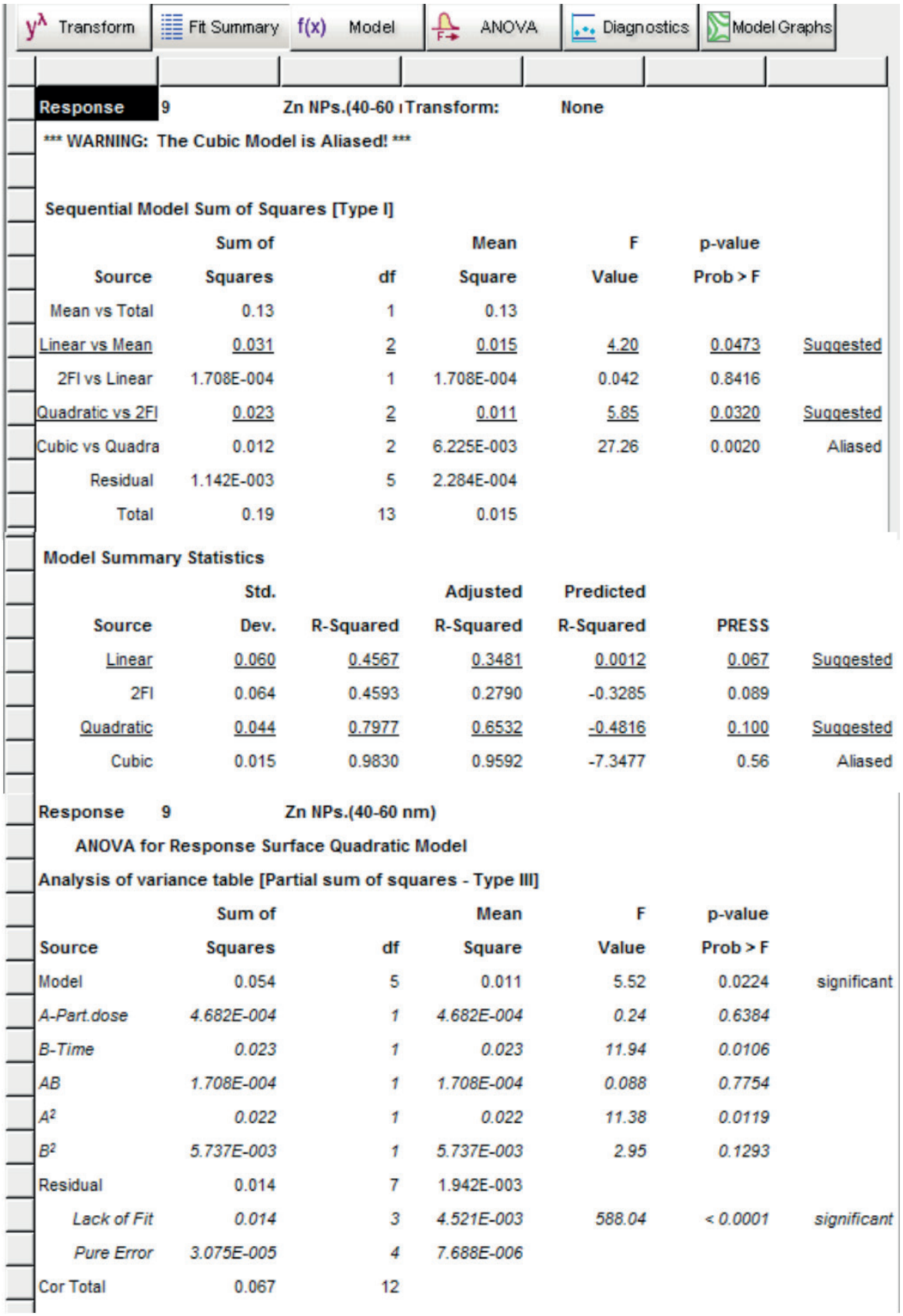
According to the statistical results provided for Zn (40-60 nm) NP, the correlation coefficient (R^2) value for appropriate Quadratic model is respectively determined as multiple R=89.31%, R^2=79.77% and adjusted R^2=65.32%. Determination of (R^2) as 79.77% shows that compatibility between observed values and foreseen values is high and applied model takes place in confidence range. The fact that multiple R value is 89.31% indicates that regression is important statistically and 10.69% of total variables cannot be explained with this model. In order to determine statistical value of this model, "Significance F" value in ANOVA test is examined. The fact that Significance F value is lower than 0.05 (0.0224) and model F value is 5.52 indicates that the model in 95% confidence range is statistically important (Figure 1 and 2).

According to mathematical modelling, it is found out that:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad \ldots\ldots(3)$$

$$Abs. = 0.18 - 0.00088\, X_1 - 0.064\, X_2 - 0.00061\, X_{12} - 0.16\, X_1^2 + 0.046\, X_2^2 \quad \ldots\ldots(4)$$

As a result of the evaluation, it is concluded that among selected parameters for Zn (40-60 nm), particle dose and time have an impact decreasing absorbance value. When absolute values of coefficients are examined, effect order is time and dose.



Transform | Fit Summary | Model | ANOVA | Diagnostics | Model Graphs

Response 9 Zn NPs.(40-60 ı **Transform:** None

*** WARNING: The Cubic Model is Aliased! ***

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	0.13	1	0.13			
Linear vs Mean	0.031	2	0.015	4.20	0.0473	Suggested
2FI vs Linear	1.708E-004	1	1.708E-004	0.042	0.8416	
Quadratic vs 2FI	0.023	2	0.011	5.85	0.0320	Suggested
Cubic vs Quadra	0.012	2	6.225E-003	27.26	0.0020	Aliased
Residual	1.142E-003	5	2.284E-004			
Total	0.19	13	0.015			

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.060	0.4567	0.3481	0.0012	0.067	Suggested
2FI	0.064	0.4593	0.2790	-0.3285	0.089	
Quadratic	0.044	0.7977	0.6532	-0.4816	0.100	Suggested
Cubic	0.015	0.9830	0.9592	-7.3477	0.56	Aliased

Response 9 Zn NPs.(40-60 nm)

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.054	5	0.011	5.52	0.0224	significant
A-Part.dose	*4.682E-004*	*1*	*4.682E-004*	*0.24*	*0.6384*	
B-Time	*0.023*	*1*	*0.023*	*11.94*	*0.0106*	
AB	*1.708E-004*	*1*	*1.708E-004*	*0.088*	*0.7754*	
A^2	*0.022*	*1*	*0.022*	*11.38*	*0.0119*	
B^2	*5.737E-003*	*1*	*5.737E-003*	*2.95*	*0.1293*	
Residual	0.014	7	1.942E-003			
Lack of Fit	*0.014*	*3*	*4.521E-003*	*588.04*	*< 0.0001*	*significant*
Pure Error	*3.075E-005*	*4*	*7.688E-006*			
Cor Total	0.067	12				

Figure 1. Statistical table for Zn (40-60 nm) NP

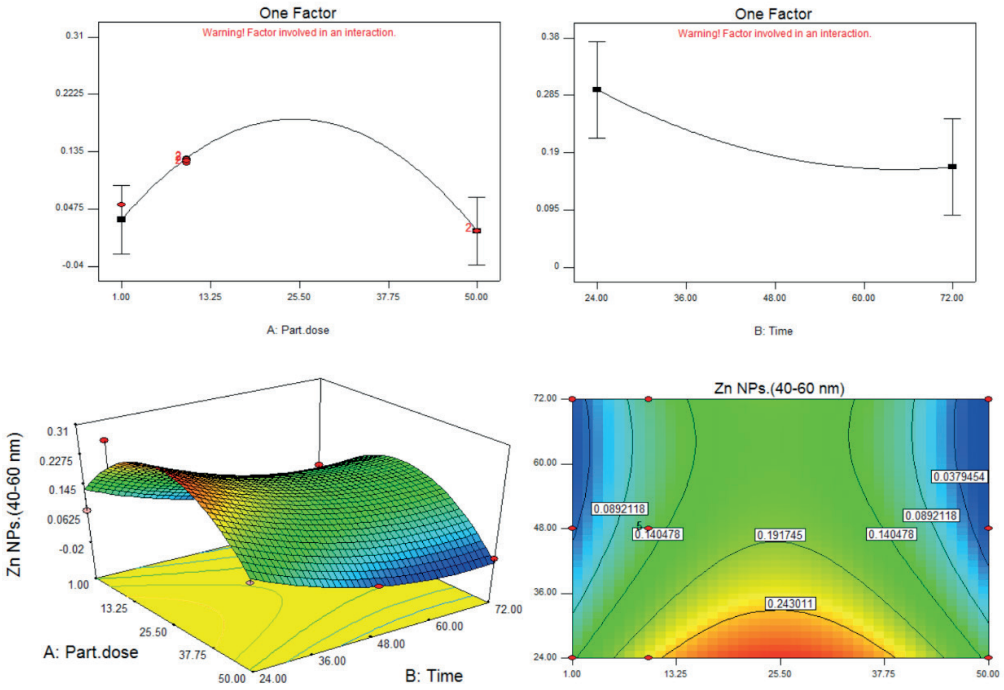


Figure 2. Graphs of statistical data for Zn NP (40-60 nm)

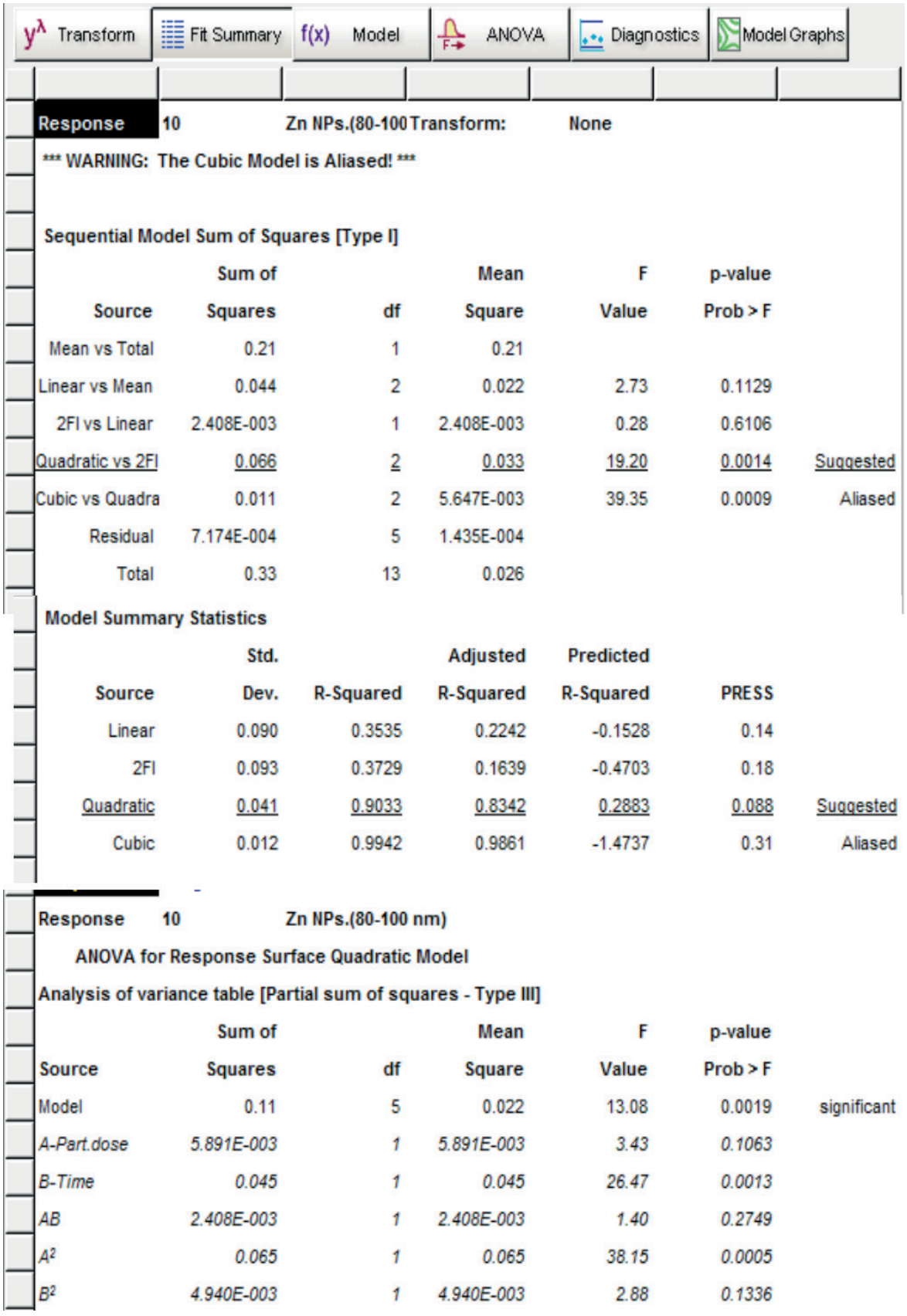
According to the statistical results provided for Zn (80-100 nm) NP, the correlation coefficient (R^2) value for appropriate Quadratic model is respectively determined as multiple R=95.04%, R^2=90.33% and adjusted R^2=83.42%. Determination of (R^2) as 90.33% shows that compatibility between observed values and foreseen values is high and applied model takes place in confidence range. The fact that multiple R value is 95.04% indicates that regression is important statistically and 4.96% of total variables cannot be explained with this model. In order to determine statistical value of this model, "Significance F" value in ANOVA test is examined. The fact that Significance F value is lower than 0.05 (0.0019) and model F value is 0.11 indicates that the model in 95% confidence range is statistically important (Figure 3 and 4).

According to mathematical modelling, it is found out that:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad \ldots\ldots(5)$$

$$Abs. = 0.31 + 0.031\ X_1 - 0.090\ X_2 - 0.023\ X_{12} - 0.28\ X_1^2 + 0.042\ X_2^2 \quad \ldots\ldots(6)$$

As a result of the evaluation, it is concluded that among selected parameters for Zn (80-100 nm), particle dose and time have an impact increasing and decreasing absorbance value, respectively. When absolute values of coefficients are examined, effect order is time and dose.



Transform | Fit Summary | Model | ANOVA | Diagnostics | Model Graphs

Response 10 Zn NPs.(80-100 Transform: None

*** WARNING: The Cubic Model is Aliased! ***

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	0.21	1	0.21			
Linear vs Mean	0.044	2	0.022	2.73	0.1129	
2FI vs Linear	2.408E-003	1	2.408E-003	0.28	0.6106	
Quadratic vs 2FI	0.066	2	0.033	19.20	0.0014	Suggested
Cubic vs Quadra	0.011	2	5.647E-003	39.35	0.0009	Aliased
Residual	7.174E-004	5	1.435E-004			
Total	0.33	13	0.026			

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.090	0.3535	0.2242	-0.1528	0.14	
2FI	0.093	0.3729	0.1639	-0.4703	0.18	
Quadratic	0.041	0.9033	0.8342	0.2883	0.088	Suggested
Cubic	0.012	0.9942	0.9861	-1.4737	0.31	Aliased

Response 10 Zn NPs.(80-100 nm)

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.11	5	0.022	13.08	0.0019	significant
A-Part.dose	*5.891E-003*	*1*	*5.891E-003*	*3.43*	*0.1063*	
B-Time	*0.045*	*1*	*0.045*	*26.47*	*0.0013*	
AB	*2.408E-003*	*1*	*2.408E-003*	*1.40*	*0.2749*	
A^2	*0.065*	*1*	*0.065*	*38.15*	*0.0005*	
B^2	*4.940E-003*	*1*	*4.940E-003*	*2.88*	*0.1336*	

Figure 3. Statistical table for Zn (80-100 nm) NP

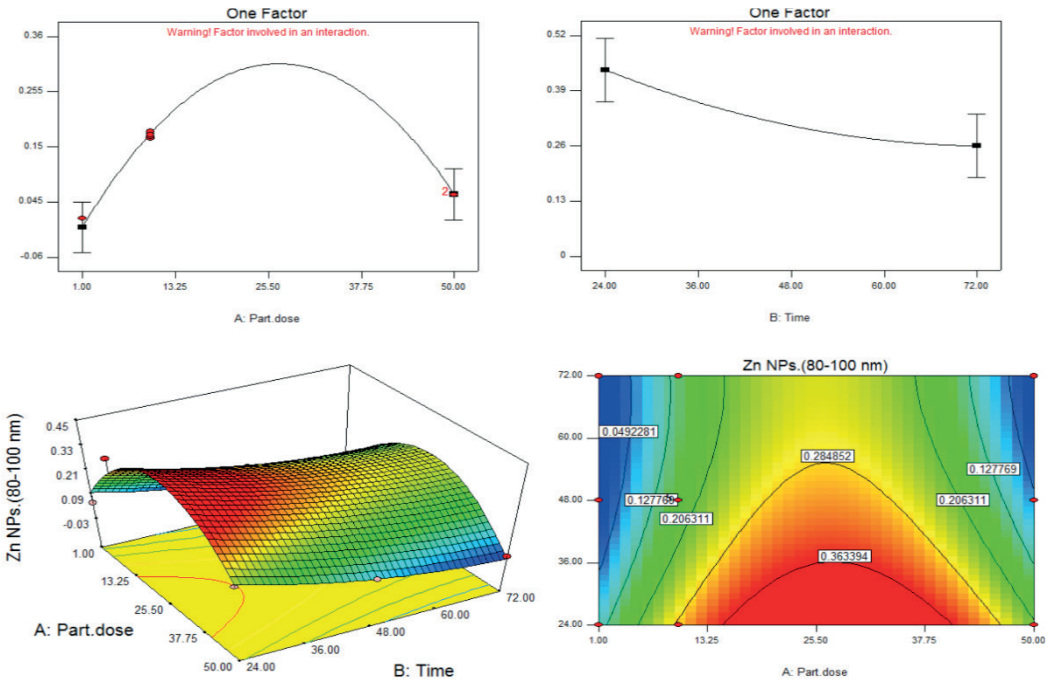


Figure 4. Graphs of statistical data for Zn NP (80-100 nm)

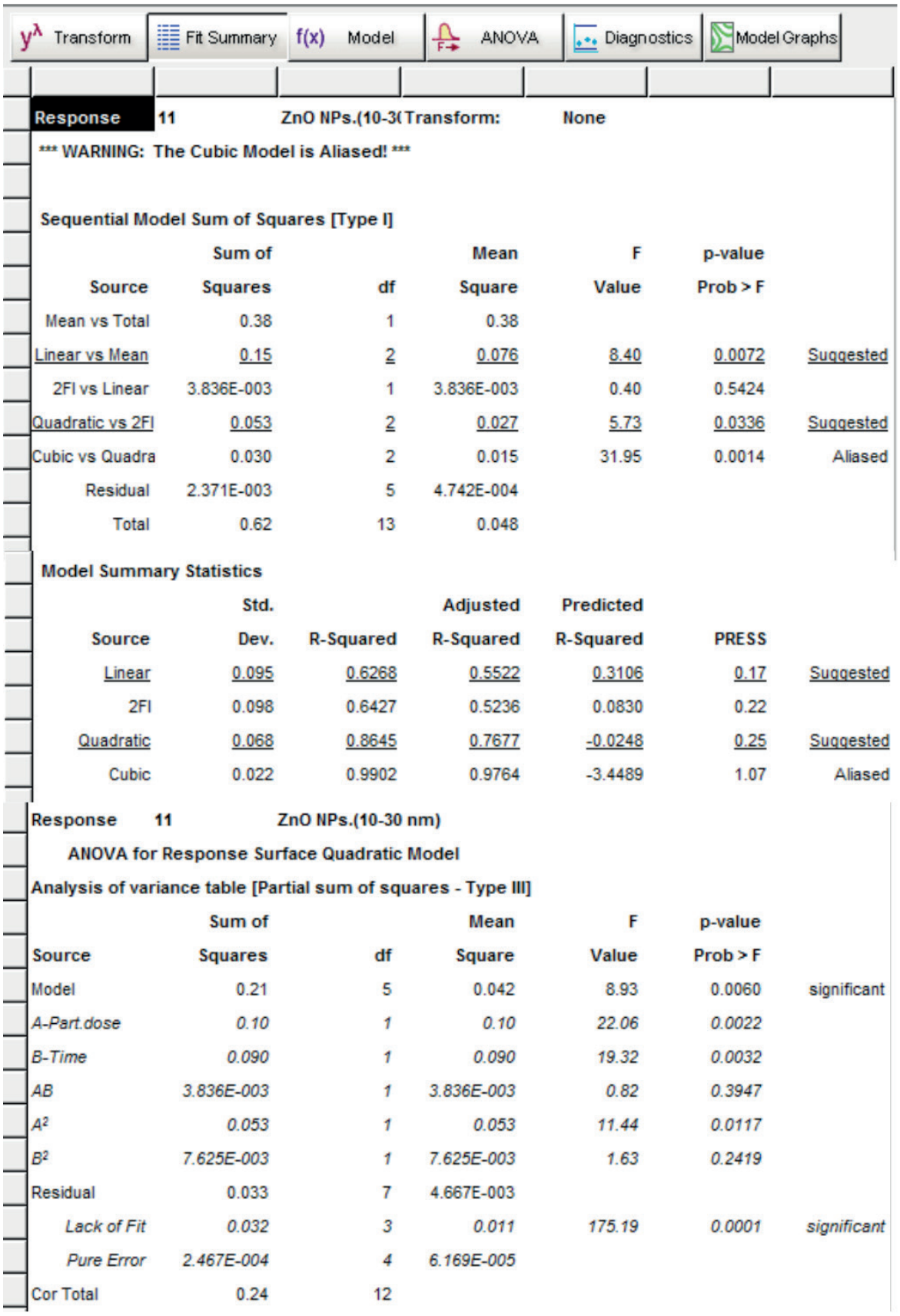
According to the statistical results provided for ZnO (10-30 nm) NP, the correlation coefficient (R^2) value for appropriate Quadratic model is respectively determined as multiple R=92.98%, R^2=86.45% and adjusted R^2=76.77%. Determination of (R^2) as 86.45% shows that compatibility between observed values and foreseen values is high and applied model takes place in confidence range. The fact that multiple R value is 92.98% indicates that regression is important statistically and 7.02% of total variables cannot be explained with this model. In order to determine statistical value of this model, "Significance F" value in ANOVA test is examined. The fact that Significance F value is lower than 0.05 (0.0060) and model F value is 8.93 indicates that the model in 95% confidence range is statistically important (Figure 5 and 6).

According to mathematical modelling, it is found out that:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad (7)$$

$$Abs. = 0.36 + 0.13\, X_1 - 0.13\, X_2 - 0.029\, X_{12} - 0.25\, X_1^2 + 0.053\, X_2^2 \quad (8)$$

As a result of the evaluation, it is concluded that among selected parameters for ZnO (10-30 nm), particle dose and time have an impact increasing and decreasing absorbance value, respectively. When absolute values of coefficients are examined, effect order of time and dose is equal.



Transform | Fit Summary | Model | ANOVA | Diagnostics | Model Graphs

Response 11 ZnO NPs.(10-3(Transform: None

*** WARNING: The Cubic Model is Aliased! ***

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	0.38	1	0.38			
Linear vs Mean	0.15	2	0.076	8.40	0.0072	Suggested
2FI vs Linear	3.836E-003	1	3.836E-003	0.40	0.5424	
Quadratic vs 2FI	0.053	2	0.027	5.73	0.0336	Suggested
Cubic vs Quadra	0.030	2	0.015	31.95	0.0014	Aliased
Residual	2.371E-003	5	4.742E-004			
Total	0.62	13	0.048			

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.095	0.6268	0.5522	0.3106	0.17	Suggested
2FI	0.098	0.6427	0.5236	0.0830	0.22	
Quadratic	0.068	0.8645	0.7677	-0.0248	0.25	Suggested
Cubic	0.022	0.9902	0.9764	-3.4489	1.07	Aliased

Response 11 ZnO NPs.(10-30 nm)

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.21	5	0.042	8.93	0.0060	significant
A-Part.dose	*0.10*	*1*	*0.10*	*22.06*	*0.0022*	
B-Time	*0.090*	*1*	*0.090*	*19.32*	*0.0032*	
AB	*3.836E-003*	*1*	*3.836E-003*	*0.82*	*0.3947*	
A^2	*0.053*	*1*	*0.053*	*11.44*	*0.0117*	
B^2	*7.625E-003*	*1*	*7.625E-003*	*1.63*	*0.2419*	
Residual	0.033	7	4.667E-003			
Lack of Fit	*0.032*	*3*	*0.011*	*175.19*	*0.0001*	*significant*
Pure Error	*2.467E-004*	*4*	*6.169E-005*			
Cor Total	0.24	12				

Figure 5. Statistical table for ZnO (10-30 nm) NP

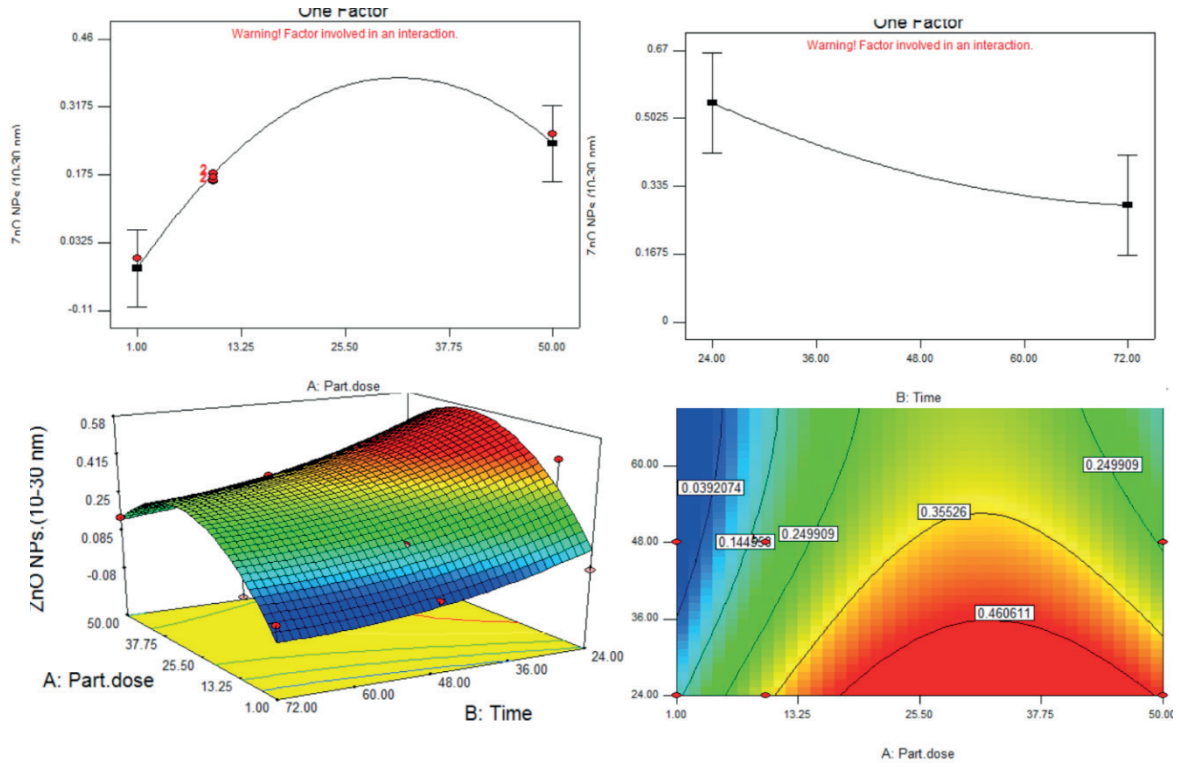


Figure 6. Graphs of statistical data for ZnO (10-30 nm) NP

As a conclusion, considering all statistical data; It was concluded that maximum effect was observed between 24-30 hours and 25 ppm absorbance concentration was more effective. Adsorption value decreased in all NPs under periods of 24 hours and above 36 hours. Similarly, the effect was reduced when the absorbance dose was low and too high. When all these data are considered, it is concluded that the effect changes according to NP type and size and the change is meaningful.

Intensive and possible misuse of nanoscale materials is one of the greatest threats to the environment and all living things. In this study, important results were obtained on the establishment of control mechanisms for uses of NP.

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