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Modeling to predict the cytotoxicity of SiO_2 and TiO_2 nanoparticles

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ABSTRACT: The objective of the current study was to design a suitable model to predict the cytotoxicity induced by SiO_2 and TiO_2 nanoparticles in different conditions using computational models. To achieve this, we employed various statistical approaches such as linear regression, as well as artificial neural networks and support vector machine (non-linear models). The effective input parameters of the SiO_2 nanoparticles were particle size, particle concentration, and cell exposure time. In the case of the TiO_2 nanoparticles, the particle size and concentration served as input variables. Cell viability was considered the output response for both nanoparticles. The modeling was performed using both linear and non-linear methods. In addition, an external validation analysis was conducted to evaluate the predictability of the models by splitting the data into training and test data. The best models to predict cell viability were the models developed by artificial neural network. The results of this investigation indicate that non-linear models could be superior to linear models in predicting cell viability for SiO_2 and TiO_2 nanoparticles.

KEYWORDS: Artificial neural network; cytotoxicity; modeling; nanoparticles

1. INTRODUCTION

A great variety of novel nanoparticles and their industrial applications raise concerns about their potential toxicity [1, 2]. Due to the expensive and time-consuming nature of the empirical assessments of the cytotoxicity of nanoparticles, it is favorable to use computational methods in order to predict the potential toxicity and environmental impact of such materials [3-5]. Recently, chemometric methods, including linear models like multiple linear regression (MLR) and non-linear models like artificial neural network (ANN) and support vector machine (SVM), have been widely used. MLR, the most common form of linear regression analysis, is a statistical approach for modeling the relationship between activity and independent descriptors [6, 7]. In addition to MLR, non-linear models such as the ANN and SVM approaches were investigated for both nanoparticles. They can be successfully applied in various fields, especially in pharmaceutical processes, where experimental information is available. In short, ANN takes input data in an input layer, computes the relationships between them in hidden layer(s), and finally the neurons generate an output layer based on the weighted sum of all inputs [8-10]. SVM is based on statistical learning theory, which consists of a training phase with associated input and output values. It creates a hyperplane in a multidimensional space to map the input related to the activity onto a higher-dimensional feature space by the kernel function. SVM was used to model the physicochemical property and activity of the drugs [11-13].

The employment of such models in nanoscience not only reduces the assessment costs but also helps in designing safe nanomaterial. This issue is very critical in nanotechnology because of the lack of comparable, consistent, and publicly accessible toxicity data [14,15]. However, few studies have focused on the application of a chemometrics-based model in the prediction of the physicochemical, biological, and fate properties (cytotoxicity) of nanomaterial [16, 17]. Cordeiro and his coworkers [18, 19] in 2014 have been reported the toxicity prediction of nanoparticles by different analysis methods, such as the linear discriminant analysis, for evaluating the safety of nanoparticles.

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In the current study, the cell viability of human embryonic kidney cells (HEK293) and mouse macrophages (Ana-1 cells), when exposed to the SiO_2 and TiO_2 nanoparticles, respectively, were considered as two data sets. Herein, the MLR, ANN, and SVM models were applied to predict the cytotoxicity of the SiO_2 and TiO_2 nanoparticles.

2. RESULTS AND DISCUSSION

2.1. Development of linear model for the training set

For the SiO_2 nanoparticles, the effects of three parameters (concentration, size, and exposure time), and for the TiO_2 nanoparticles, that of two parameters (concentration and size) on the cytotoxicity have been reported in the literature [20, 21]. There is a good linear correlation between the cell viability and the concentration of SiO_2 nanoparticles. Furthermore, the exposure time has a significant effect on cell viability in both the studied cases. The linear regression analysis of cytotoxicity versus the studied parameters confirms the mathematical relations among the parameters. The developed multiple linear regression models with the corresponding statistical parameters for SiO_2 (the training data set) is (Model 1):

$$\text{Cell viability (\%)} = 122.118 - 0.188 \times \text{Concentration} - 1.261 \times \text{Time} \quad \text{(Eq. 1)}$$

$N=30 \quad R^2=0.773 \quad F=46.0 \quad p<0.001 \quad RMSE=12.55$

and for TiO_2 (the training data set) is (Model 2):

$$\text{Cell viability (\%)} = 91.935 + 0.002 \times \text{Concentration} \quad \text{(Eq. 2)}$$

$N=14 \quad R^2=0.852 \quad F=31.5 \quad p<0.001 \quad RMSE=2.56$

Size of nanoparticles has an important role in cell viability. In a previous study by Baharifar and Amani on chitosan/streptokinase nanoparticles, they showed that the most important factor in determining the particles' toxicity is size of nanoparticles [22]. However, size (in the studied range of nanoparticle size) has no significant statistically effect ($p>0.1$) on cell viability. The statistical parameters (after excluding the particle size of the nanoparticles) related to the linear regression models are the squared correlation coefficient (R^2), the Fisher test statistic (F) that show the fit of a regression equation to the training set data and the corresponding probability values (p-value) [23], and the root mean square error (RMSE). In the developed linear regression models for both nanoparticles, the probability values (p-value) for each selected variable are less than 0.05. These verify the significant effect of the selected parameters (concentration and time of SiO_2 nanoparticles, and concentration of TiO_2 nanoparticles) on the cytotoxicity of the nanoparticles for both case studies.

2.2. Development of non-linear models for the training set

Two ANN models with different hidden layers and transfer functions were identified as the best models for both data sets. Introducing particle size could provide improved statistical parameters for the developed model—i.e. Model 3 (Table 1). However, the external validation results (Figure 1 and 2) show that the established models (model 2) without the size of the nanoparticles have a prediction capability that is comparable to the model including this parameter.

Table 1. Developed ANN models and the corresponding statistical parameters for training set of SiO_2 and TiO_2 nanoparticles.

No.	Developed network	No. Input Layer	No. hidden Layer 1	No. hidden Layer 2	No. output Layer	R^2	RMSE
			SiO_2				
2	RBF[a]	2	10	0	1	0.95	5.95
3	MLP[b]	3	10	7	1	0.99	2.25
			TiO_2				
2	MLP	1	5	0	1	0.92	1.91
3	GRNN[c]	2	11	2	1	0.96	1.44

[a]Radial-based function, [b]Multiple layer perceptron, [c]Generalized regression neural network

Prediction of cytotoxicity nano-sized metal oxides by ANN has been reported in the literature. In a recent study by Fjodorova et al, the cytotoxicity prediction of metal oxide nanoparticles by ANN was studied with χ-metal electronegativity (EN) by Pauling scale and composition of metal oxides [24]. Moreover, Baharifar and Amani in a study on cytotoxicity of albumin-loaded chitosan nanoparticles and modeling by ANN showed that concentrations of initial materials are the most important factors which may affect the cell viability [25].

In addition, SVM models were developed by the training sets, and the optimized and statistical parameters of the models have been listed in Table 2. However, evaluation of the prediction capability by test set was not acceptable (Figure 1 and 2).

Table 2. Developed SVM model and the corresponding statistical parameters for training sets of SiO_2 and TiO_2 nanoparticles.

No.	C	ε	γ	R^2	RMSE
SiO_2					
4	126	0.1	0.01	0.75	13.2
5	131	0.1	0.05	0.86	10.4
6	36	0.1	0.1	0.86	10.3
TiO_2					
4	35	0.3	0.1	0.87	2.4
5	36	0.1	0.5	0.97	1.2
6	20	0.1	1	0.98	1.2

C, ε and γ are parameters of SVM.

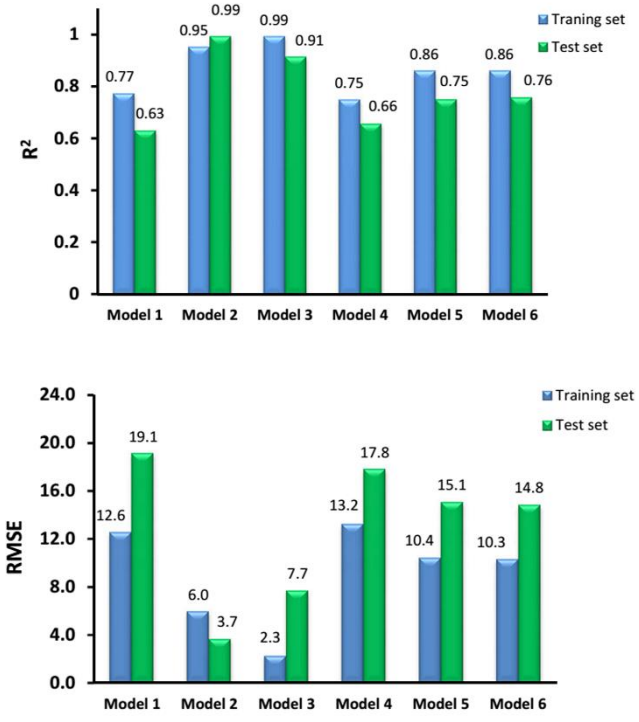

Figure 1. R^2 (correlation between experimental and calculated values) of training and test sets (top figure) and RMSE values (down figure) of developed linear and non-linear models for SiO_2 nanoparticles (model 1 is a linear model i.e. MLR, model 2-6 are non linear models (model 2, 3 :ANN and model 4, 5 and 6: SVM).

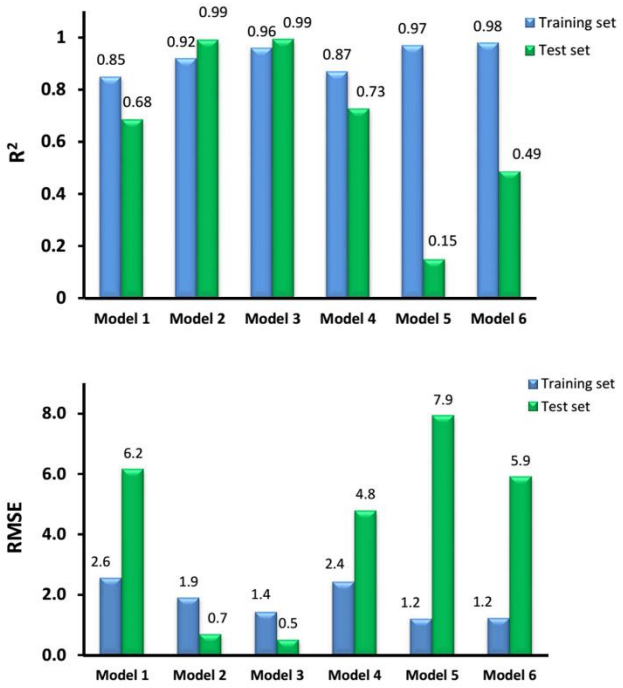

Figure 2. R^2 (correlation between experimental and calculated values) of training and test sets (top figure) and RMSE values (down figure) of developed linear and non-linear models for TiO2 nanoparticles (model 1 is a linear model i.e. MLR, model 2-6 are non linear models (model 2, 3 :ANN and model 4, 5 and 6: SVM).

2.3. External validation and comparison between the developed linear and non-linear models

External validation is a critical stage that should be examined to ensure the prediction capability of a model. The over-fitting and incapability of a model to predict external data is common in the development of non-linear models. The development of models and the evaluation of the statistical parameters for training models are not sufficient in modeling studies. In the predictive models, external validation is essential to ensure confidence in their predictability and generalizability [26, 27]. A necessary parameter to assign an external validation of the computational methods is R^2. However, R^2 is not enough to ensure the predictability of a model. Another method that is used to confirm the validity of the computational methods is the calculation and comparison of model errors (i.e. RMSE) for the training and test sets [28]. In this report, R^2 and a comparison between the RMSE of the training and test values were employed as the appropriate statistical parameters to evaluate the predictability of all the models developed using linear and non-linear statistical methods. Figures 1 and 2 show the R^2 and RMSE values of the established models for the training and test sets. The R^2 of all the models for the test set of both nanoparticles were more than 0.6 (a critical criterion for the validity of a model), except for Models 5 and 6 for the TiO_2 nanoparticles (over-fitting). Furthermore, the slightly significant differences between the RMSE of the training and test sets, especially in the ANN models, verify the validity of the developed models. These results indicate the best model for both nanoparticles obtained through ANN. The significant improvement in predictive capability exhibited by the ANN and SVM models confirms the importance of non-linear modeling in predicting the cytotoxicity of nanoparticles with different characteristics. Similiar studies confirm the importance of new statistical methods and sofware such as Monte Carlo technique [29] and CORAL [30], respectively, for prediction of cytotoxicity for metal oxide nanoparticles.

3. CONCLUSION

In this report, the applied linear and non-linear approaches to design the predictive models for cell viability (%) exposed to SiO_2 and TiO_2 nanoparticles, as well as their predictability and performance, were compared with each other. Based on the obtained results, it can be concluded that size has no significant effect on cell viability against the concentration and time of cell exposure. Also, non-linear modeling could be employed as a more appropriate method for predicting the cytotoxicity of nanoparticles. Moreover, external

validation is necessary to check the over-fitting problem and the selection of the appropriate parameters, especially in non-linear modeling.

4. MATERIALS AND METHODS

Data on the cell viability of cultured human embryonic kidney cells and cultured mouse macrophages measured by an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay were taken from the literature [20, 21] and divided randomly into training (30 data for SiO_2, 14 data for TiO_2) and test sets (10 data for SiO_2, 7 data for TiO_2) (Table 3 and Table 4).

Table 3. Cell viability (%) of cultured human embryonic kidney cells exposed to SiO_2 nanoparticles at various conditions reported data by Wang et al [20].

Order	Particle size (nm)	Concentrations (mg/L)	Exposure time (h)	Cell viability (%)
1*	20	25	0 (no exposure)	100.0
2	20	50	0 (no exposure)	99.8
3	20	100	0 (no exposure)	100.0
4	20	200	0 (no exposure)	100.0
5	20	25	12	101.7
6*	20	50	12	98.4
7	20	100	12	96.2
8	20	200	12	95.9
9	20	25	24	89.6
10	20	50	24	70.2
11*	20	100	24	34.7
12	20	200	24	33.0
13	20	25	36	78.3
14	20	50	36	67.2
15	20	100	36	34.0
16*	20	200	36	27.1
17*	20	25	48	75.0
18	20	50	48	62.8
19	20	100	48	26.7
20	20	200	48	20.3
21*	50	25	0 (no exposure)	100.0
22	50	50	0 (no exposure)	100.0
23	50	100	0 (no exposure)	100.1
24	50	200	0 (no exposure)	99.9
25	50	25	12	101.7
26	50	50	12	102.1
27	50	100	12	104.2
28*	50	200	12	100.9
29*	50	25	24	92.7
30	50	50	24	74.6
31	50	100	24	56.4
32	50	200	24	39.8
33	50	25	36	78.4
34*	50	50	36	74.6
35	50	100	36	56.1
36	50	200	36	36.1
37	50	25	48	71.1
38	50	50	48	70.8
39	50	100	48	53.0
40*	50	200	48	26.7

*Test set.

Table 4. Cell viability (%) of cultured mouse macrophages cells exposed to TiO_2 nanoparticles at various conditions reported data by Zhang et al [21].

Order	Particle size (nm)	Concentrations (mg/L)	Cell viability (%)
1	5	12.5	96.7
2	5	25	94.2
3*	5	50	90.9
4	5	100	84.2
5	5	200	81.7
6	5	400	77.6
7*	5	600	76.8
8	25	12.5	91.8
9	25	25	89.2
10	25	50	86.7
11*	25	100	81.8
12*	25	200	78.4
13	25	400	74.3
14*	25	600	70.9
15	100	12.5	93.4
16	100	25	91.0
17	100	50	89.2
18*	100	100	85.1
19	100	200	81.7
20	100	400	78.4
21*	100	600	77.2

*Test set

MLR, the most common form of linear regression analysis, is a statistical approach to model the relationship between activity and independent descriptors [6]. Moreover, a neural network was generated to make the non-linear relationship between input neurons (particle sizes, particle concentrations and cell exposure time) and output neurons (cytotoxicity) using various functions, including the generalized regression neural network (GRNN), the radial-based function (RBF), and the multiple-layer perceptron (MLP), along with different numbers of hidden neurons via the STATISTICA 7 software. The prediction of cell viability (%) of studied nanoparticles was assessed by another non-linear model, namely SVM. Capacity parameter (C), ε and γ are parameters of SVM which optimized. C is a regularization parameter that adjusts maximizing, the distance from the hyperplane to training set data points and ε is related to noise in the data [11, 12]. They were optimized by cross-validation method using STATISTICA 7 software.

The statistical parameters of the training set—i.e. the coefficient of determination between the experimental and prediction data (R^2) and the root mean square error (RMSE) calculated as:

$$R^2 = 1 - \frac{\sum(\text{exprimental value} - \text{calculated value})^2}{\sum(\text{exprimental value} - \text{mean of exprimental value})^2} \quad \text{(Eq. 3)}$$

$$RMSE = \frac{\text{Square error}(SE)}{N} = \frac{\sum(\text{experimental value} - \text{calculated value})^2}{N} \quad \text{(Eq. 4)}$$

The development of models and evaluation of the statistical parameters for the training model are not sufficient in modeling studies. In the predictive models, external validation is essential for the confidence of their predictability and generalizability [26, 27]. Therefore, the validity of the developed model was checked by the test set.

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