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# Safety assessment of biological nanofood products via intelligent computer simulation

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**Abstract.** Emerge of nanotechnology impacts all aspects of humans' life. One of important aspects of the nanotechnology and nanoparticles (NPs) is in the food production industry. The safety of such foods is not well recognized and producing safe foods using nanoparticles involves delicate experiments. In this study, we aim to incorporate intelligent computer simulation in predicting safety degree of nanofoods. In this regard, the safety concerns on the nano-foods are addressed considering cytotoxicity levels in metal oxides nanoparticles using adaptive neuro-fuzzy inference system (ANFIS) and response surface method (RSM). Three descriptors including chemical bond length, lattice energy and enthalpy of formation gaseous cation of 15 selected NPs are examined to find their influence on the cytotoxicity of NPs. The most effective descriptor is selected using RSM method and dependency of the toxicity of these NPs on the descriptors are presented in 2D and 3D graphs obtained using ANFIS technique. A comprehensive parameters study is conducted to observe effects of different descriptors on cytotoxicity of NPs. The results indicated that combinations of descriptors have the most effects on the cytotoxicity.

**Keywords:** biological activity; cytotoxicity, nano-food; neuro-fuzzy system; response surface method

## 1. Introduction

Nano-ingredients in foods exist in the form of natural nanostructures including proteins and carbohydrates (Habibi *et al.* 2016, 2018a, b, 2019b, d, e, Ebrahimi *et al.* 2019a, Esmailpoor Hajilak *et al.* 2019, Pourjabari *et al.* 2019, Safarpour *et al.* 2019a). However, engineered nano materials (ENMs) have been used in producing foods and food related products and they have the most safety relevant concerns in literature. With advancements in nano-technology (Adamian *et al.* 2020, Al-Furjan *et al.* 2020a, b, Li *et al.* 2020b, Liu *et al.* 2020b, Zare *et al.* 2020, Dai *et al.* 2021b, Habibi *et al.* 2021, He *et al.* 2021, Huang *et al.* 2021a, Liu *et al.* 2021b, Zhang *et al.* 2021a) and emersion of novel higher quality structures, there have been inclinations to utilize nano-materials in packaging food products (Safarpour *et al.* 2018, 2019b, 2020, Habibi *et al.* 2017, 2019a, c, Alipour *et al.* 2020, Ebrahimi *et al.* 2020a, Ghazanfari *et al.* 2020, Chen *et al.* 2022). Further, the nano-scale metal oxides were employed in drug delivery applications. In addition, some foods contain ENMs. These application of nano-particles (NPs) in close connections with human rose questions regarding effects of such materials on the health. Thus, it is extremely important to answer the questions on the healthiness of such foods and materials before further using them.

The cytotoxicity and biological activity of NPs is the subject of many researches (Puzyn *et al.* 2011, Gajewicz *et al.* 2014, Hajizadeh *et al.* 2019). Buglak *et al.* (2019) reviewed the most recent studies on the cytotoxicity of nano-scale particles which used structure-activity relationships (SAR) methods in prediction of level of toxicity of NPs. These methods used emis analytical methods to predict the possible level of toxicity, biological activity and other parameters and quantify them. Investigating publications of several nano-scale materials, they concluded that quasi-SAR methods is the most appropriate method in evaluating toxicity of such structures. Gajewicz *et al.* (2015) used combined theoretical QSAR and experimental method to evaluate toxicity of 18 different metal oxide NPs. They compared toxicity effects on the two prokaryotic and eukaryotic systems. The QSAR method is useful method which can predict the most effective chemical component in desired aim. Further, this method can be used alongside with computational chemistry to eliminate non-effective parameters or descriptors in a component. Thus, it has been widely used in place of experiments. The final results and chemicals selected in these methods to perform a specific task should be validated and adjusted with experimental work. Therefore, these methods considerably reduce experimental costs and time. Martirosyan and Schneider (Martirosyan and Schneider 2014) investigated the publication on the toxicity of food related NPs and discussed safety issues in the most common engineered nano-materials used in food production. Some ENMs are accumulated in different human organs and some in some special condition this accumulation can be amplified. In a valuable paper, Puzyn and coworkers (Puzyn *et al.* 2011) used experimental method to evaluate toxicity of 17 metal oxide commonly found in foods. They concluded that $\Delta H_{Me+}$, representing the enthalpy of

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formation of a gaseous cation, are the most important descriptor of the metal oxides molecules. Furthermore, they present a linear correlation equation using QSAR to predict other metal oxide toxicity based on $\Delta H_{Me+}$. It is concluded that ZnO, CuO, NiO and CoO had the most cytotoxicity in metal oxides. Due to lack of experimental data on metal oxide, many other papers utilized the data given in (Puzyn *et al*. 2011) paper to investigate other NPs (Fjodorova *et al*. 2017). In the present study, we also use these set of data to correlate some other descriptors with cytotoxicity using novel artificial intelligence (AI) methods. Tortella *et al*. (2020) reviewed papers on the toxicity level of silver nanoparticles and its effect on the living organisms. They reported that the AgNPs has detrimental effects on different organisms. Arias *et al*. (2018) and Burk *et al*. (2018) presented novel descriptors to address the toxicity of Fe-doped and ZnO nanoparticles. Arias *et al*. (2018) presented the researches on the iron oxide NPs in drug delivery systems. It was discussed that due to existence of diverse methods in synthesis of such NPs with different size, shape and dispersion comparison of reported results is not directly possible. In a recent study, Papadiamantis *et al*. (2020) used in silico methods to find 7 effective descriptors in metal oxide among 77 descriptors under consideration. These selected descriptors were claimed to be most effective in cytotoxicity. The used experimental data from (Zhang *et al*. 2012).

Using artificial intelligence (Shariati *et al*. 2012, 2016a, b, 2019, 2020d, e, f, g, h, i, j, 2021a, b), numerical modeling (Ebrahimi *et al*. 2019b, c, Hashemi *et al*. 2019, Moayedi *et al*. 2019, 2020a, b, Mohammadgholiha *et al*. 2019, Mohammadi *et al*. 2019, Ebrahimi *et al*. 2020b, Habibi *et al*. 2020, Oyarhossein *et al*. 2020, Shariati *et al*. 2020a, b, Shokrgozar *et al*. 2020), and machine learning (ML) methods (Al-Furjan *et al*. 2020d, e, f, g, h, i, l, n, o, Liu *et al*. 2020a, Wang *et al*. 2020, Zhou *et al*. 2020, Dai *et al*. 2021a, Guo *et al*. 2021a, Shao *et al*. 2021, Wu and Habibi 2021) in cytotoxicity have attracted many researchers in the field of nanotechnology and biology. Singh *et al*. (2020) comprehensively reviewed the publications on the toxicity prediction in the biomedical materials with emphasis on NPs. They presented a roadmap to use AI methods in nano-cytotoxicity. They also discussed the major concerns on the quality of publications in the application of AI methods in nano-toxicity. Cytotoxicity of silver nanoparticles were investigated by Liu *et al* (2021c) using two models of machine learning method namely random forest and decision tree. They claimed that using machine learning algorithm guides the future studies for improved designs of experiments. Fjodorova *et al*. (2017) utilized previously measured toxicity level of metal oxide presented by (Puzyn *et al*. 2011) and discussed other effective descriptors in metal oxide. They generalized their QSAR model to other 72 metal oxide in periodic table. The results indicated that using artificial neural network (ANN) method could be a robust procedure in determining toxicity of metal oxide. Kar *et al*. (2021) employed seven different ML algorithms to identify the most toxic metal oxide nanoparticles. They concluded that with increase in the electron number which indicates electron transfer between living organs and NPs resulting in release of free radicals. Subramanian and Palaniappan (2021) utilized a neural network (NN) with one hidden layer to predict the cytotoxicity of metal oxide NPs. Their model had >96% accuracy in predicting cytotoxicity of these type of NPs. Bushueva *et al*. (2019) focused on the toxicity of two nanoparticles PbO and CuO on human fibroblasts. It was shown that the concentration of both NPs are in direct correlation with cell damage. The response surface method was utilized the cytotoxicity of these NPs mathematically. There are many other publications using machine learning models to predict toxicity of metal oxides NPs (Jones *et al*. 2016, Sizochenko *et al*. 2019, Yu *et al*. 2021, Ji *et al*. 2022). The anti-bacterial effects of nano-composites reinforced with silver, copper and zinc oxides NPs were investigated by (Dehghani *et al*. 2021) on two common type bacteria. They presented a regression model with high accuracy to predict anti-bacterial characteristics of such composites. Moradi *et al* (2022) reviewed publications on the food additives based on NPs. Bumbudsanpharoke and Ko (2015) investigated the literature to present migration of nanoparticles from packages into food and possible hazards of these NPs for humans.

In the present study, the safety concerns on the nano-foods are addressed considering cytotoxicity levels in metal oxides nanoparticles used in food production using adaptive neuro-fuzzy inference system (ANFIS) and response surface method (RSM). In this regard, three descriptors of 15 selected NPs including chemical bond length, lattice energy and number of electrons in the last layer of the metal atom are examined. The most effective descriptor is selected using RSM method and dependency of the toxicity of these NPs on the descriptors are presented in 2D and 3D graphs obtained using ANFIS technique.

## 2. Cytotoxicity of nanoparticles in food productions

Cytotoxicity in food productions can be regarded as effects of nanoparticle on the living cells in human organs. The main ways of confronting human with NPs due to food production is due to nanoparticles used in packaging in the role of antibacterial agents and in food ingredients and also in drug delivery NPs (Wu *et al*. 2017, 2018, 2021a, b, Zhong *et al*. 2022). The main NPs in the food productions are $SiO_2$, $TiO_2$, $AgO$ and $CuO$. The short-term and long-term effects of these NPs as well as other NPs are not clearly recognized and researches on these materials is going on. The toxicity effects of the NPs is not only due to their chemical composition. Size and structure of NPs can also be toxic to human organs because of excessive accumulations in particular organ. On the other hand, structure of nanoparticles and ratio of surface to volume is another important factor in toxicity (Lai *et al*. 2021, Ren *et al*. 2022, You *et al*. 2022, Zhang *et al*. 2022b). Due to the vast physicochemical descriptors in toxicity, simulations become extremely complicated and also there are factors which is not realized deeply to add into simulations. Thus, we must rely on the experimental studies on the NPs and try to find correlation between different descriptors and cytotoxicity of NPs. In this regard, the importance of mathematical methods manifest itself.

Table 1 Metal oxides NPs characteristics from Refs (Puzyn *et al.* 2011, Haynes 2014, Gagne and Hawthorne 2020)

| Metal Oxide | Bond Length [Å] | Lattice Energy [kJ/mol] | Charge of Metal Cation | Number of Metal atoms | E/B [kJ/mol] | $\Delta H_{Me+}$ [kcal/mol] | $\log\left(\frac{1}{EC_{50}}\right)$ [mol/l] |
|---|---|---|---|---|---|---|---|
| $CoO$ | 2.130 | 3837 | 2 | 1 | 1918.5 | 601.8 | 3.51 |
| $NiO$ | 2.084 | 3908 | 2 | 1 | 1954 | 596.7 | 3.45 |
| $CuO$ | 1.943 | 4135 | 2 | 1 | 2067.5 | 706.25 | 3.2 |
| $ZnO$ | 1.952 | 4142 | 2 | 1 | 2071 | 662.44 | 3.45 |
| $ZrO_2$ | 2.221 | 11188 | 4 | 1 | 2797 | 1,357.66 | 2.15 |
| $SnO_2$ | 2.110 | 11807 | 4 | 1 | 2951.75 | 1,717.32 | 2.01 |
| $TiO_2$ | 1.940 | 12150 | 4 | 1 | 3037.5 | 1,575.73 | 1.74 |
| $La_2O_3$ | 2.290 | 12452 | 3 | 2 | 2075.33 | 1,017.22 | 2.87 |
| $Y_2O_3$ | 2.264 | 12705 | 3 | 2 | 2117.5 | 837.15 | 2.87 |
| $SiO_2$ | 1.540 | 13125 | 4 | 1 | 3281.25 | 1,686.38 | 2.2 |
| $In_2O_3$ | 2.142 | 13928 | 3 | 2 | 2321.33 | 1,271.13 | 2.81 |
| $Fe_2O_3$ | 2.015 | 14309 | 3 | 2 | 2384.83 | 1,408.29 | 2.29 |
| $V_2O_3$ | 2.007 | 15096 | 3 | 2 | 2516 | 1,097.73 | 3.14 |
| $Cr_2O_3$ | 2.060 | 15276 | 3 | 2 | 2546 | 1,268.70 | 2.51 |
| $Al_2O_3$ | 1.870 | 15916 | 3 | 2 | 2652.67 | 1,187.83 | 2.49 |

## 3. Materials and methods

### 3.1 Metal oxides nano-particles

The common metal oxide nano-particles used in food industry is listed in Table 1. The properties of the lattice and bond between oxygen and other properties are extracted using Refs. (Puzyn *et al.* 2011, Haynes 2014, Gagne and Hawthorne 2020). We aim to find a correlation physico-chemical between properties of the metal oxides and 50% reduction in bacteria viability $\log(1/EC_{50})$ for these NPs (Jia *et al.* 2021, Zhang *et al.* 2021b, 2022a, Cheng *et al.* 2022, Duan *et al.* 2022). In this regard, 6 descriptors bond length, lattice energy, charge of metal cation, number of metal atoms in molecule, lattice energy per bond and enthalpy of formation of cation were investigated to fine the most influential descriptors.

### 3.2 Response Surface Method (RSM)

Finding the most effective parameters from an experimental study is commonly performed using response surface methodology (Wong 1985). In this method, the data are fitted with 2-degree polynomial and the influence of the factors are determined using a chart called Pareto chart. The important parameters in Table 1 were found after several runs of the RSM method. It was concluded that the bond length, lattice energy per bond in molecule $E/B$ and enthalpy of formation of gaseous cation $\Delta H_{Me+}$ are the most effective descriptors.

### 3.3 Adaptive Neuro-Fuzzy Inference System (ANFIS)

Although RSM method provide a simple and convenient equations to determine the most effective descriptor as well as an effective optimization method, the accuracy of the model is highly dependent on the provided data (Hashemi *et al.* 2019, Al-Furjan *et al.* 2020c, j, k, m, Bai *et al.* 2020, Cheshmeh *et al.* 2020, Li *et al.* 2020a, Lori *et al.* 2020, Najaafi *et al.* 2020, Shariati *et al.* 2020c, Xiong *et al.* 2020, Guo *et al.* 2021b, Liu *et al.* 2021a). Thus in this study, we employ another effective and high efficiency method adaptive neuro-fuzzy inference system or ANFIS (Jang 1993) to construct a model for prediction of toxicity of NPs. This method is based on the fuzzy logic in which a linguistic description of the factors is converted to numeric values using membership functions. The value obtained of the membership function is normalized and final results is the sum of the all membership values influenced by the descriptors to find toxicity of the NPs (Fan *et al.* 2022, Luo *et al.* 2022, Michael *et al.* 2022, Wang *et al.* 2022a, Wang *et al.* 2022b, Yang *et al.* 2022, Zheng *et al.* 2022). The neural network backward propagation method is utilized in this fuzzy system to adopt the coefficient of the membership function as well as the influence factors of the original value of the descriptor. A detail discussion with comparative examples could be found in original article by (Jang 1993) and many other references. The member ship function utilized in this study in generalized bell-shaped function with the following relation (Ma *et al.* 2021, Zhao *et al.* 2021, Hou *et al.* 2021, Huang *et al.* 2021b, c, Jiao *et al.* 2021, Liu *et al.* 2021d, Moradi *et al.* 2021, Xu *et al.* 2021, Yu *et al.* 2022):

$$f(x,a,b,c)=\frac{1}{1+\left|x-c\right|^{2b}} \qquad (1)$$

where the constants $a$, $b$ and $c$ determines the general shape and position of the MF curve. Three examples of similar MFs with different centers are depicted in Fig. 1.

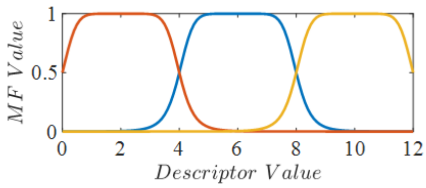

Fig. 1 Generalized bell-shaped membership functions with $a = 2,\ b = 4$ and $c = 2, 6, 10$ in Eq. (1)

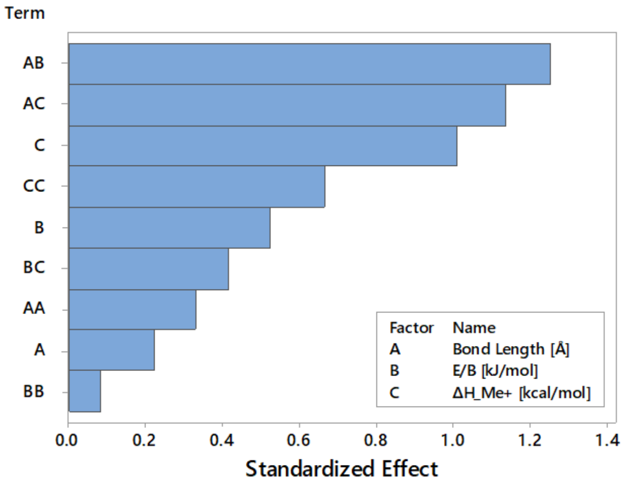

Fig. 2 Pareto chart of effective descriptor on 50% reduction in bacteria viability using RSM

## 4. Results and discussion

### 4.1 RSM results

In Fig. 2, the influence of different descriptors bond length (A), lattice energy per bond in molecule $E/B$ (B) and enthalpy of formation of gaseous cation $\Delta H_{Me+}$ (C) and their product combinations are depicted in a Pareto diagram. It is seen that among A, B and C factors the C descriptor or $\Delta H_{Me+}$ has the most effect on the toxicity of the NPs used in food industry. This result is in harmony with other studies (Puzyn *et al*. 2011). However, it is found that this descriptor solely is not the major factors in toxicity. Indeed, product value of bond length (A) and $E/B$ (B) have more effect than others. Thus, it should be given attention to combinations of several factors in considering such cases.

The accuracy of the fitted quadrilateral RSM model is shown in Fig. 3 as its residual in different situations. The charts show that the residuals of the actual measured values of $\Delta H_{Me+}$ with the values calculated from obtained RSM model lay between $-0.3 < Res. < 0.4$ which could be considered significant in comparison to actual maximum value of 3.51. On the other hand, the squared correlation coefficient $R^2 = 0.79$ which is shown that, although acceptable to some extent, we need more accurate and robust model to predict toxicity of NPs.

The analysis of variance of different descriptor is given in Table 2 in detail. The higher F-value and lower P-value determine the most effective parameters in the toxicity of NPs. As can be recognized, the combinations of bond length and E/B as well as combination of bond length and $\Delta H_{Me+}$ are the most important factors to consider.

Table 2 Analysis of variance

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Model | 9 | 4.15437 | 0.461597 | 6.86 | 0.024 |
| Linear | 3 | 2.04532 | 0.681773 | 10.14 | 0.014 |
| $Bond\ Length$ [Å] | 1 | 0.0033 | 0.003301 | 0.05 | 0.833 |
| $E/B$ [kJ/mol] | 1 | 0.0182 | 0.018205 | 0.27 | 0.625 |
| $\Delta H_{Me+}$ [kcal/mol] | 1 | 0.06862 | 0.068617 | 1.02 | 0.359 |
| Square | 3 | 0.10971 | 0.03657 | 0.54 | 0.673 |
| $(Bond\ Length)^2$ | 1 | 0.00739 | 0.007394 | 0.11 | 0.754 |
| $(E/B)^2$ | 1 | 0.0005 | 0.000496 | 0.01 | 0.935 |
| $(\Delta H_{Me+})^2$ | 1 | 0.02986 | 0.029862 | 0.44 | 0.535 |
| 2-Way Interaction | 3 | 0.11163 | 0.03721 | 0.55 | 0.668 |
| $Bond\ Length$ [Å] $\times E/B\ [kJ/mol]$ | 1 | 0.10512 | 0.105117 | 1.56 | 0.267 |
| $Bond\ Length$ [Å] $\times \Delta H_{Me+}\ [kcal/mol]$ | 1 | 0.08673 | 0.086725 | 1.29 | 0.308 |
| $E/B\ [kJ/mol] \times \Delta H_{Me+} [kcal/mol]$ | 1 | 0.01152 | 0.011524 | 0.17 | 0.696 |
| Error | 5 | 0.33632 | 0.067264 | | |
| Total | 14 | 4.49069 | | | |

Table 3 optimized values of selected descriptors to minimize $\log\left(\frac{1}{EC_{50}}\right)$

| Bond Length [Å] | $E/B\ [kJ/mol]$ | $\Delta H_{Me+}\ [kcal/mol]$ | $\log\left(\frac{1}{EC_{50}}\right)$ |
|---|---|---|---|
| 1.68 | 2856 | 1623 | 1.74 |

The merit of RSM method is to find the optimum condition based on the fitted model. Although the fitted model does not have acceptable accuracy, but it gives a general behavior of the model. To minimize the amount of toxicity based on the $\log\left(\frac{1}{EC_{50}}\right)$ the RSM model gives the values in Table 3. As seen, the optimized values are not minimum nor maximum values of the descriptor showing indirect correlation between these factors.

Effects of different descriptors on the toxicity of the model using RSM model is given in Figs. 4 to 6. As seen in Fig. 4, in high values of $E/B$s and low values of bond lengths the amount of toxicity is reduced with increase in enthalpy of formation of gaseous cation $\Delta H_{Me+}$. However, with decrease in $E/B$ and increase in bond length the behavior of the toxicity is changed and in specific points it started to increase with increase in $\Delta H_{Me+}$. In general, with decrease in lattice energy of nanoparticles used in nano-food industry it is safe to say that the toxicity of the NPs in terms of biology activity decreases. On the other hand, increase in bong length has positive effect on the toxicity in lower $\Delta H_{Me+}$ while caution must be given to high $\Delta H_{Me+}$ where increase in bond length dramatically increase toxicity.

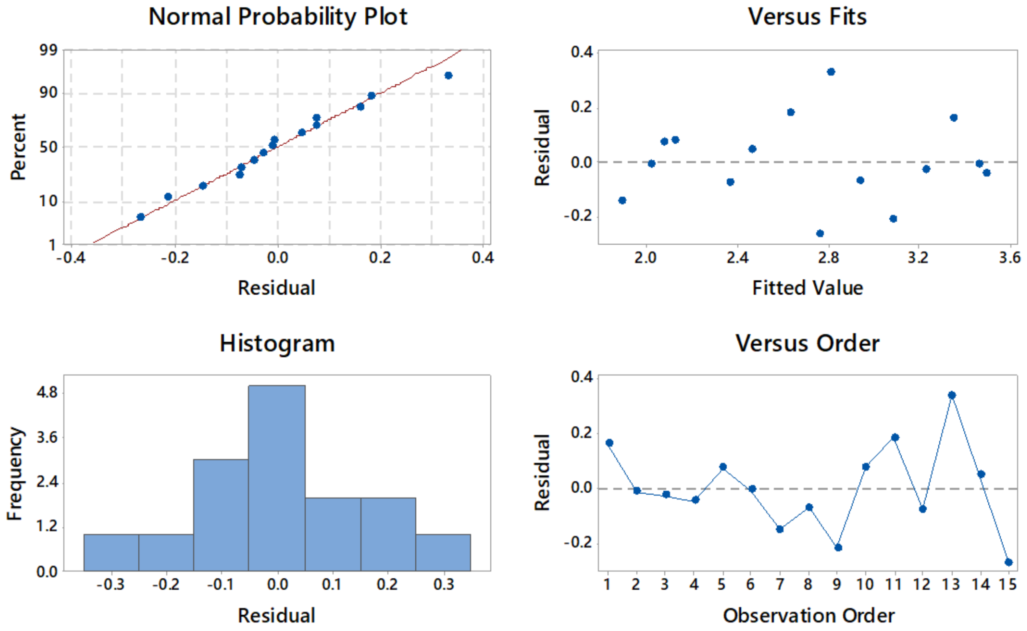

Fig. 3 Residuals of the fitted quadrilateral model in comparison with actual values

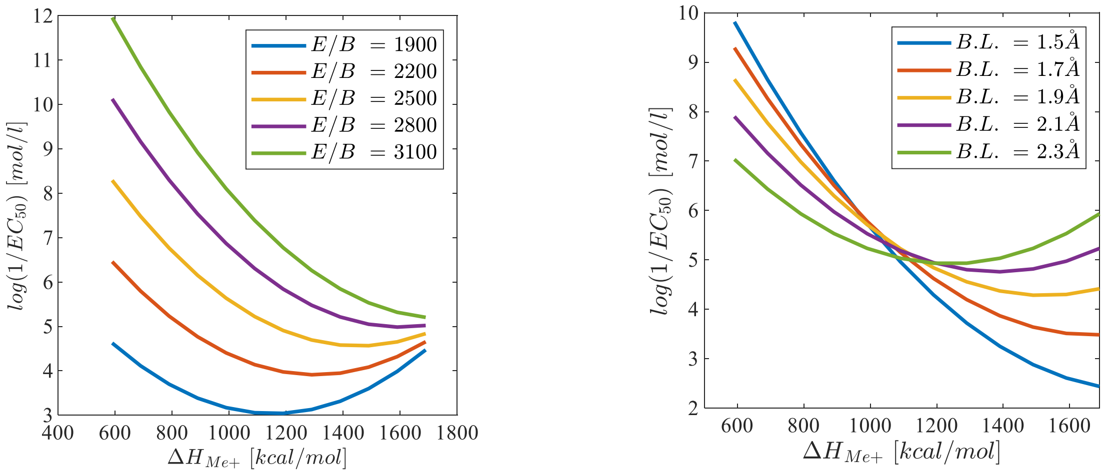

Fig. 4 Toxicity of the NPs as function of $\Delta H_{Me+}$ for different values of bond length and lattice energy per bond using RSM; The left figure in $B.L. = 2.0$ Å and the right figure is depicted for $E/B = 2500\ kJ/mol$

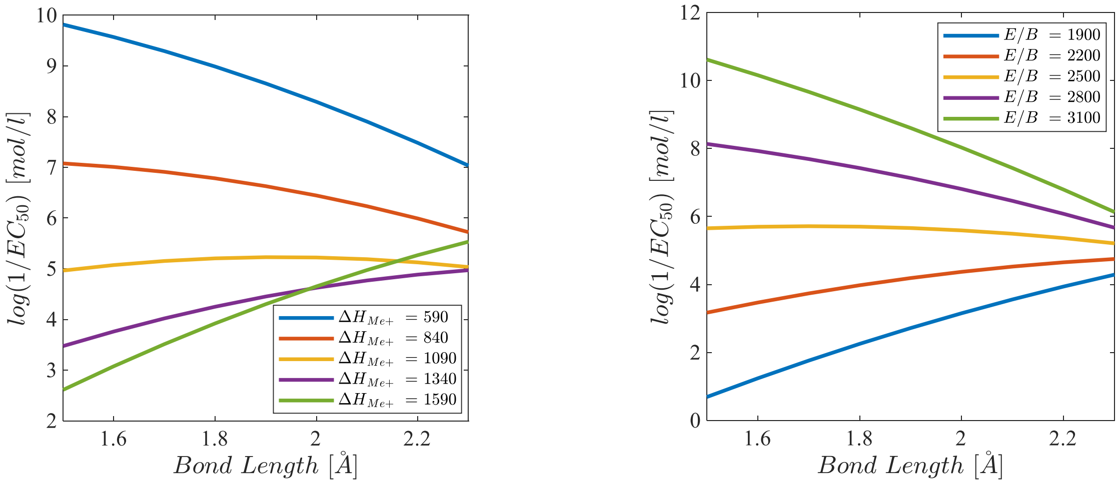

Fig. 5 Toxicity of the NPs as function of bond length for different values of $\Delta H_{Me+}$ and lattice energy per bond using RSM; The left figure in $E/B = 2500\ kJ/mol$ and the right figure is depicted for $\Delta H_{Me+} = 1000 kcal/mol$

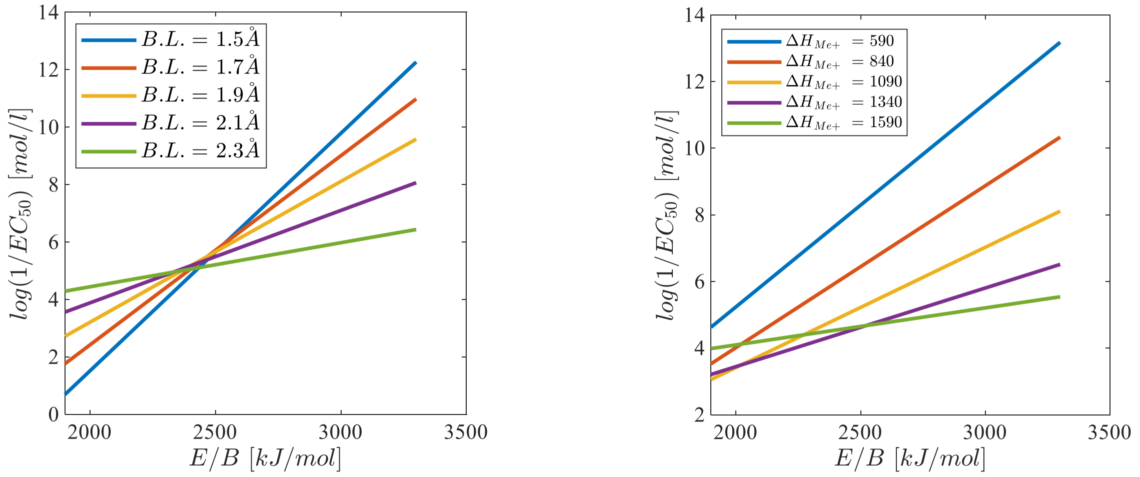

Fig. 6 Toxicity of the NPs as function of lattice energy per bond for different values of $\Delta H_{Me+}$ and bond length using RSM; The right figure in $B.L. = 2.0$ Å and the left figure is depicted for $\Delta H_{Me+} = 1000 kcal/mol$

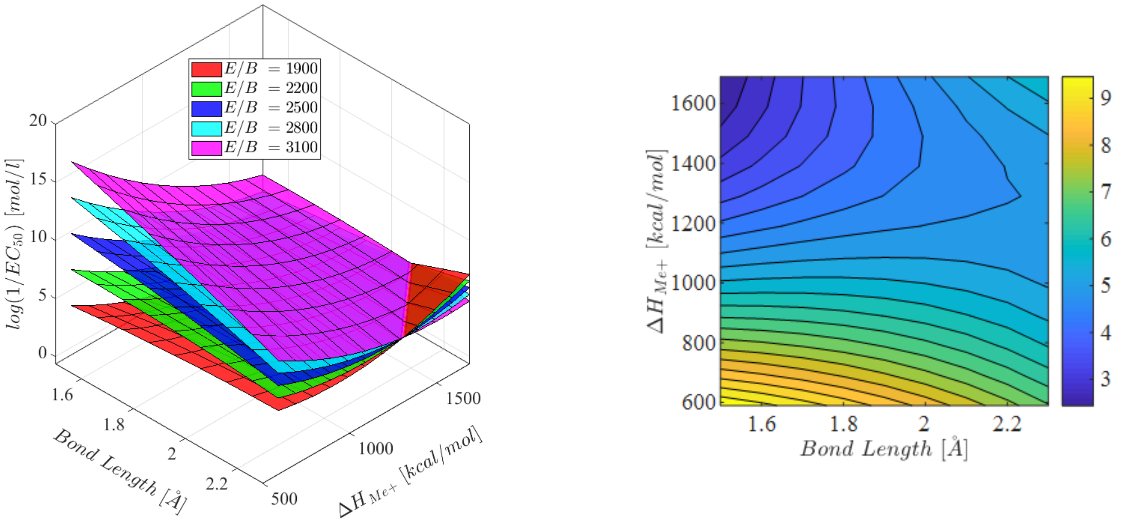

Fig. 7 Cytotoxicity of NPs as function of different descriptors using RSM

The effect of bond length on the biological activity of the NPs is shown in Fig. 5. As can be observed the effect of bond length is highly dependent on other two descriptors i.e. lattice energy and $\Delta H_{Me+}$. Base on the values of the $E/B$ and enthalpy of formation of cation the bond length effect can be detrimental or positive or even it has no effect on the toxicity. Specifically in $\Delta H_{Me+} = 1090$ $kcal$/mol and $E/B = 2500$ $kJ/mol$ the effects of bond length can be neglected. On the other hand, at lower values of the bond length, change in the other descriptors values have more effects on the biological activity and toxicity of the NPs.

The effect of the $E/B$ descriptor is linear as shown in Fig. 6. It is concluded from the fitted model in which the coefficient of $\left(\frac{E}{B}\right)^2$ equals to zero. Moreover, in all conditions of other descriptors, increase in lattice energy results in increase in toxicity of the NPs and this increase is more pronounce in lower values of bond length and $\Delta H_{Me+}$where the slope of the line become intense.

For more comprehension of the effects of different descriptors 3D and contour graphs of level of toxicity is depicted in Fig. 7. It is observed that there is a common intersection line in surfaces with different lattice energy. However, this line is not the minimum of the surfaces. Furthermore, in the contour plot for $E/B = 2500 kJ/mol$ it is seen that lower value of bond length and higher values of $\Delta H_{Me+}$ is more desirable to reduce toxicity.

### 4.2 Neuro-fuzzy analysis

The network of ANFIS is depicted in Fig. 8. The model is employed in Matlab software using Neuro-Fuzzy Designer App. As seen, there are three numbers of inputs representing 3 selected descriptors bond length, lattice energy per bond and enthalpy of formation of gaseous cation. To each of these 3 inputs, three membership function of Eq. (1) are assigned which determine the low, medium and high values of descriptors. These membership functions is in “inputmf” layer. Another layer in this network is the governing rules of the layer in which all the values of the membership functions are added together to have effects of all descriptors. Moreover, these values are

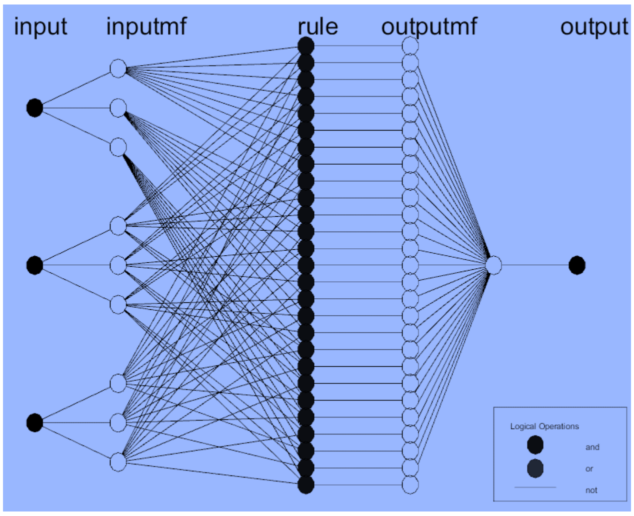

Fig. 8 Neuro-fuzzy network to predict toxicity of NPs based on three descriptors as inputs

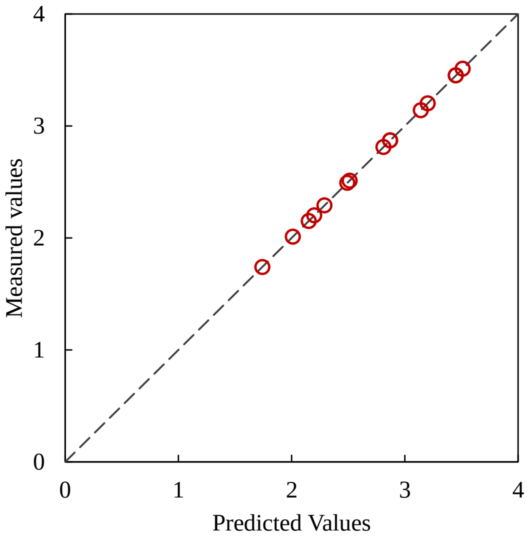

Fig. 9 Perfection of NPs cytotoxicity using ANFIS

normalized to be used in the next layer “outputmf” where defuzzification occurs. At the last layer all the effects are collected to find the cytotoxicity of the descriptors.

The efficiency of the ANFIS method is shown in Fig. 9 where prediction accuracy of the network can be realized with $R^2 = 0.9999$. This accuracy postulates the high potential application of this method.

In Fig. 10, the effects of three descriptors of NPs on toxicity are depicted using surface meshes. As seen, the prediction of ANFIS is different from RSM and ANFIS has more reliable results. The RMS is regression method that in case of this study has $R^2_{RSM} = 0.79$ which is low values compared to $R^2_{ANFIS} = 0.9999$. Thus, the results of ANFIS must be considered for any conclusions.

Effects of different descriptors on the toxicity of the model using ANFIS model is given in Figs. 11 to 13. As seen in Fig. 11, in all values of $E/B$s and bond lengths the amount of toxicity is reduced with increase in enthalpy of formation of gaseous cation $\Delta H_{Me+}$. In general, with decrease in lattice energy of nanoparticles used in nanofood industry it is safe to say that the toxicity of the NPs in terms of biology activity decreases. On the other hand, increase in bong length has positive effect on the toxicity in lower $\Delta H_{Me+}$. The results are in harmony with the outcomes of RSM.

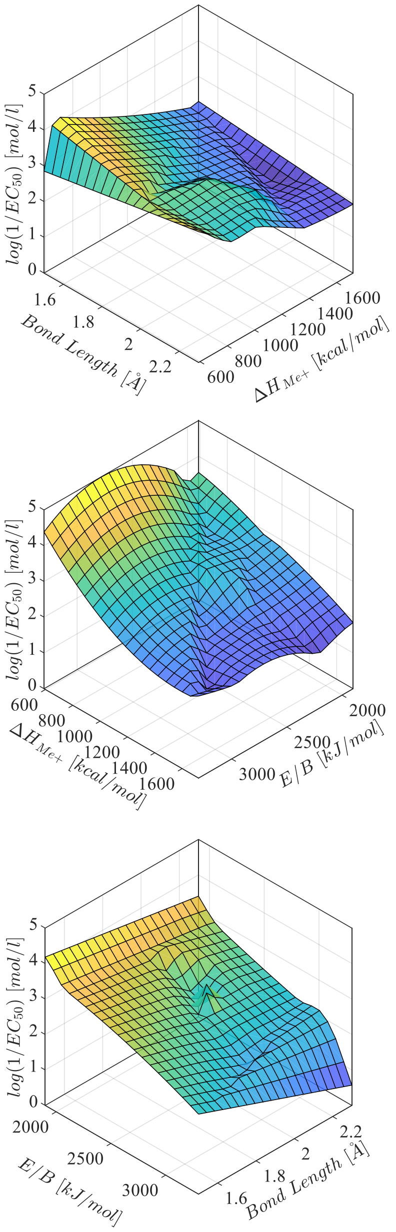

Fig. 10 Cytotoxicity of NPs as function of different descriptors using ANFIS

The effect of bond length on the biological activity of the NPs is shown in Fig. 5. As can be observed, increase in bond length slightly reduces cytotoxicity of NPs regardless of values of lattice energy and enthalpy of formation of gaseous cation. However, in some case a small fluctuations is seen which can be related to numerical error in fitting curves.

Effect of the lattice energy per bond is demonstrated in Fig. 13. In contrast to RSM method, these graphs show that increasing $E/B$ may result in decrease, increase and no effect on the cytotoxicity of the NPs. As shown for different

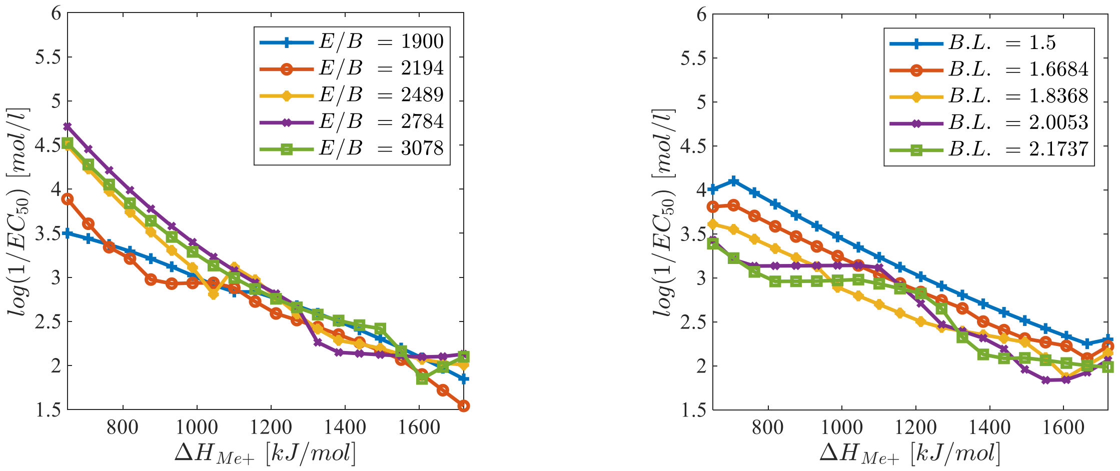

Fig. 11 Toxicity of the NPs as function of $\Delta H_{Me+}$ for different values of bond length and lattice energy per bond using ANFIS; The left figure in $B.L. = 2.0$ Å and the right figure is depicted for $E/B = 2500\ kJ/mol$

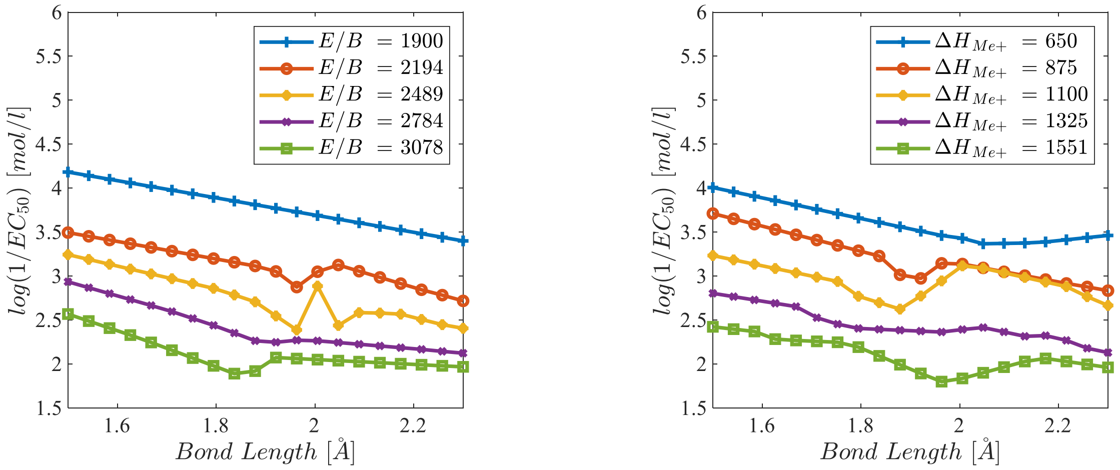

Fig. 12 Toxicity of the NPs as function of bond length for different values of $\Delta H_{Me+}$ and lattice energy per bond using ANFIS; The left figure in $E/B = 2500\ kJ/mol$ and the right figure is depicted for $\Delta H_{Me+} = 1000 kcal/mol$

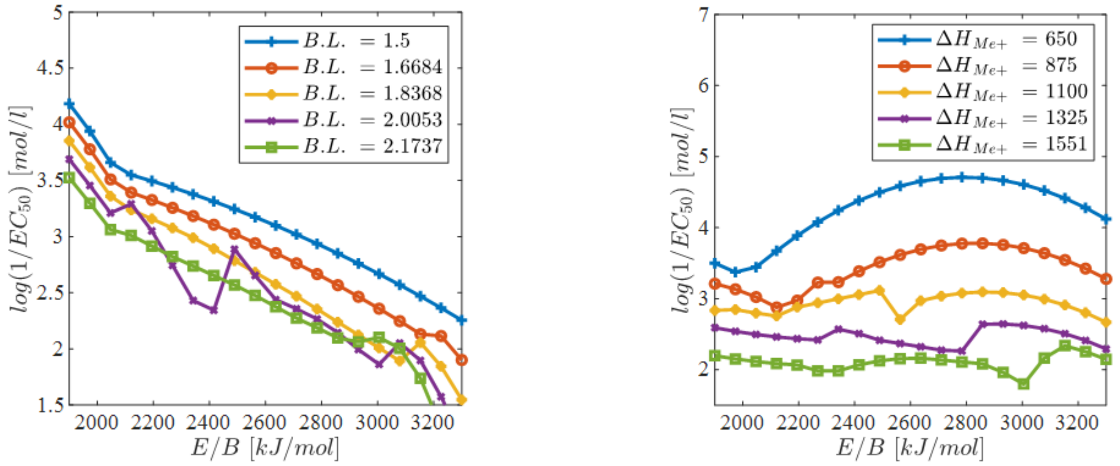

Fig. 13 Toxicity of the NPs as function of lattice energy per bond for different values of $\Delta H_{Me+}$ and bond length using ANFIS. The right figure in $B.L. = 2.0$ Å and the left figure is depicted for $\Delta H_{Me+} = 1000 kcal/mol$

values of bond length and $\Delta H_{Me+} = 1000 kcal/mol$, increase in $E/B$ leads to decrease in toxicity. Thus, changes in bond length has slight effect on toxicity of NPs in different $E/B$ as was also seen in Fig. 12. However, change in $\Delta H_{Me+}$ results in fluctuations in toxicity curves versus $E/B$. In high values of $\Delta H_{Me+}$, increase in $E/B$

has not significant effect on the cytotoxicity, but in lower values, increase in lattice energy at first results in increase in toxicity and afterwards decrease it.

## 5. Conclusions

In the present study, we incorporated intelligent computer simulation of ANFIS and RSM in predicting safety degree of nanofoods. In this regard, the safety concerns on the nano-foods were addressed considering cytotoxicity levels in metal oxides nanoparticles using experimental data and adaptive neuro-fuzzy inference system (ANFIS) and response surface method (RSM). Three descriptors including chemical bond length, lattice energy and enthalpy of formation gaseous cation of 15 selected NPs were examined to find their influence on the cytotoxicity of NPs. The most effective descriptor was determined using RSM to be combination of bond length and lattice energy and dependency of the toxicity of these NPs on the descriptors were presented in 2D and 3D graphs obtained using ANFIS technique. A comprehensive parameters study is conducted to observe effects of different descriptors on cytotoxicity of NPs. The main results of the study can be encapsulated in the followings:

- The toxicity of CoO, ZnO and NiO are highest values among investigated NPs and $TiO_2$ has the lowest value of toxicity.
- With decrease in lattice energy of nanoparticles used in nano-food industry it is safe to say that the toxicity of the NPs in terms of biology activity decreases.
- Increase in bond length slightly reduces cytotoxicity of NPs regardless of values of lattice energy and enthalpy of formation of gaseous cation.
- Changes in bond length has slight effect on toxicity of NPs in different $E/B$.
- In high values of $\Delta H_{Me+}$, increase in $E/B$ has not significant effect on the cytotoxicity, but in lower values, increase in lattice energy at first results in increase in toxicity and afterwards decrease it.

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