Classification of Inhaled Silver Nanoparticles Cytotoxicity on Alveolar Epithelial Cells using Artificial Neural Network

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***Abstract*—Due to the significant increase in the usage of silver nanoparticles in different industries, the need of knowing their potential hazard on humans is an appealing area of research. Workers who work in such fields are the most threatened by the harmful effects that may occur due to the consistent exposure to Ag-NPs during work hours, where inhalation is considered the most common way by which they are exposed to airborne Ag-NPs. In order to avoid adverse effects of Ag-NPs we should have a clear insight about their possible toxicity on humans, and the factors that may trigger it. Classifying the toxicity of Ag-NPs as high toxicity, medium toxicity, and low toxicity will make it easier for us to distinguish between silver nanoparticles, and take the appropriate action toward those with high toxicity levels. This research proposes a multilayer feed forward classification artificial neural network for the purpose of classifying inhaled silver nanoparticles cytotoxicity levels on workers' alveolar epithelial cells. Artificial neural networks are well known for their ability of classification, where they can classify different data under the right category after being trained for several times. Actually, the choice of artificial neural networks to classify the cytotoxicity of Ag-NPs came from their ability to classify the cytotoxicity of new engineered Ag-NPs that may be manufactured in the future. Experiments were performed to evaluate the proposed method of classification. Results showed that the proposed method is reliable in the classification process.**

***Index Terms*—Silver nanoparticles, alveolar epithelial cells, neural network, matrix, size, shape, and surface charge.**

I. Introduction

Nanoparticles are defined by the US National Nanotechnology Initiative as particles having at least one diameter measuring 100 nm or less [11]. Silver based Nanoparticles are increasingly being used in different products like textiles, contraceptives, and cosmetics due to their antibacterial effect [6], which increases the chance by which we are exposed to them. Ag-NPs were reported to cause dermal and cosmetic toxicity. Also, they showed a harmful effect on male reproduction system [15]. However, this research concentrates on the inhaled silver nanoparticles cytotoxicity because inhalation is the most dangerous way by which we are exposed to Ag-NPs; this is because what we breathe can be circulated to all body organs by the circulatory system. Many Studies conducted in this field concentrated on one parameter only, like shape-dependent toxicity or, size-dependent toxicity, there are no study analyzing an integrated set of chemical and physical parameters at the same experiment. It is important to consider more than one parameter like surface charge, shape, and size while assessing the toxicity of silver nanoparticles in order to know the relationship between them and any resulted toxicity; otherwise the area of silver nanoparticles toxicity or any other nanoparticles toxicity may remain vague. In this paper, the classification of inhaled silver nanoparticles cytotoxicity on workers' alveolar epithelial cells is proposed to be done by classification artificial neural network with back-propagation learning algorithm. The proposed neural network showed a reliable method for classifying the cytotoxicity of Ag-NPs based on the standards conducted in this research. It is expected to serve well in classifying the cytotoxicity currently, and in the future. The aim is to make the classification process easier, and faster. This neural network may be able to predict the cytotoxicity levels without the need of in vivo and in vitro experiments, where it will act as an expert that is able to predict the correct class after being trained for several times.

II. Inhaled Silver Nanoparticles Cytotoxicity On Alveolar Epithelial Cells

Respiratory tract is a main portal of airborne nanoparticles. A study done by Negender Panyala, Eladia Mendez, and Josef Havel about studying the properties of Ag-NPs and their adverse effects stated that inhaled airborne silver nanoparticles do not interfere with the microcilliary process in the respiratory tract. Instead, they can easily pass to the alveolar region and deposit there where they can interact with epithelial cells generating surface radicals and reactive oxygen species (ROS) which are even more toxic. The same study reported that silver and silver salts can easily interact with cellular environment in a human's body, and they can spread all over our bodies, where depositions of silver substance were found in different organisms in the body like spleen, liver, and even kidneys [15]. In addition to the previous information, alveoli are well known for their very large

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surface area [21], meaning that the chance of having an adverse effect with toxic particles including silver nanoparticles is high. Christina R. Kearns stated in his study In Vitro Toxicity of Silver Nanoparticles In Human Lung Epithelial Cells that silver nanoparticles' adverse interaction with mitochondria in human cells has been observed, where they have been directly linked to the decrease of mitochondria activity in the cells [5]. A study titled Cytotoxicity And Inflammation In Human Alveolar Epithelial Cells Following Exposure To Occupational Levels Of Gold And Silver Nanoparticles done in 2012 aimed to show in vitro response of human A549 adenocarcinomic alveolar epithelial cells following exposure to gold (Au-NP) and silver (Ag-NP) nanoparticles at levels approximating an occupational exposure showed that occupational exposure to Au-NPs and Ag-NPs significantly increased the secretion of IL-8 which caused inflammatory response [7]. One of the most common ways by which people are exposed to silver nanoparticles is inhalation. That's why, Ag-NP have a potential impact on the deep region of the lung, specially the alveolar region. [22]

III. Which Are More Toxic Silver Nanoparticles, Or Silver Ions?

An important question that has to be answered before starting the assessment of inhaled silver nanoparticles cytotoxicity on alveolar epithelial cells is which are more toxic, silver nanoparticles, or silver ions? The answer is silver ions. A study titled Antibacterial Activity And Toxicity Of Silver – Nanosilver Versus Ionic Silver pointed out that silver ions are more toxic to human fibroblast cells than silver nanoparticles are. The study aim was to compare the cytotoxicity and the antibacterial effects of silver nanoparticles to those of silver ions. Silver nanoparticles were used with concentrations approximately equal to 1 mg/L - 3 mg/L, they showed an affective suppresses of bacterial and yeast growth with no cytotoxicity levels to fibroblast cells. However, in the case of silver ions with concentrations equal to approximately 1mg/L antibacterial and yeast growth was affectively suppressed, but with much higher levels of cytotoxicity to eukaryotic organisms including humans' fibroblast cells. The same research stated that concentrations of silver ions that lead to the death of all of the tested organisms are much lower than the toxic concentrations of silver nanoparticles [14]. Also, it has been shown that silver salts have high antibacterial and cytotoxicity reactions to bone cells than silver nanoparticles do. Silver nanoparticles had an affective antibacterial reaction but with much less cytotoxicity to the bone cells [5]. Another study done In 2009 about cytotoxicity and genotoxicity of silver nanoparticles on human cells stated that surface oxidation of silver nanoparticles which occurs when contacting with cells cultures medium or proteins existing in the cytoplasm releases silver ions that can increase the degree of cytotoxicity[16]. From this it can be concluded that the real hazard may be due to the release of silver ions through the body when being exposed to silver nanoparticles.

IV. Concepts Used.

In this research, three physical and chemical parameters are used in the process of classifying the cytotoxicity of inhaled silver nanoparticles on alveolar epithelial cells. The following are the parameters:

1) Size
2) Shape
3) Surface charge

Size and surface charge are chosen as affecting parameters because they are reported as main parameters that usually influence nanoparticles toxicity [19]. Also, it has been stated that shape plays a crucial role in affecting the toxicity of nanoparticles [4]. In addition to that, it has been pointed out that the physico-chemical properties of metallic nanoparticles (such as Ag-NP) are greatly influenced by both size and shape. [22]

V. Size-Dependent Cytotoxicity On Alveolar Epithelial Cells

This research is dedicated for studying the cytotoxicity of inhaled silver nanoparticles on alveolar epithelial cells that fall in the size range of 100 nm. The size of silver nanoparticles does affect other properties of nanoparticles in the following ways:

A. Size Effect On Silver Nanoparticles Surface Area to Volume Ratio:

A study in 2012 about toxicity affects of nanoparticles showed that the small size of nanoparticles causes them to have a larger surface area to volume ratio. As a result, more molecules of chemicals are exposed to the surface increasing the incidence of chemical reactions. So, the smaller the size the more toxic a nanoparticle may be [17].

B. Size Effect On Silver Nanoparticles Oxidative Dissolution:

In a study tiltled Size And Surface Area Dependent Toxicity Of Silver Nanoparticles In Zebrafish Embryos (*Danio Rerio*) done in 2012 by George Tuttle, it has been stated that Oxidative dissolution happens when both oxygen and proteins are in action with silver nanoparticles, resulting in the release of silver ions into biological systems. This study reported that silver nanoparticles oxidative dissolution is inversely proportional to the particle's size, because smaller silver nanoparticles have a greater surface area to mass ratio, and as a result a higher percentage of silver ions release[8].

C. Size Effect On Silver Nanoparticles Transportation Inside The Body:

A study titled Silver Nanoparticles done by Hassan Korbekandi and Siavash Iravani pointed out that the small size of all nanoparticles enables them to pass through biological membranes, and even more, they can pass through very small capillaries throughout the humans' body. [10]. Also, studies showed that silver

nanoparticles don't interface with the microcillia in the respiratory systems so they can easily reach alveolar epithelial cells, even more, they can accumulate in different organisms and tissues showing different toxicity levels. Also, they have the ability to bind to tissues causing toxic affects like cell activation, production of (ROS), and cell death [15]. In addition to the previous study, another study pointed out that particles having the size smaller than 2500 nm are able to pass the respiratory tract to the alveolar reagion [5], where they can interact with epithelial cells causing different levels of inflammations. From this, it can be concluded that the size greatly affects the cytotoxicity of inhaled silver nanoparticles on alveolar epithelial cells, where it affects surface area to volume ratio, Ag ions release, the accessibility to alveolar space, and even the transporting of silver nanoparticles throughout the entire body. In this paper, the range of nanoparticles' size (1nm-100nm) is devided into three ranges for the purpose of measuring the cytotoxicity as the following:

1) The Size range [1nm , 33.33nm] is considered the smallest possible size, and thus the highest in cytotoxicity, this is due to its large surface area to volume ratio, high levels of Ag ions release, and high mobility inside the alveolar space.
2) The size range (33,33nm,66.67nm] is considered medium size. As a result, it's expected to have a smaller surface area to volume ratio, and medium levels of Ag ions release inside the alveolar space. Thus, they are expected to have medium cytotoxicity.
3) The size range (66.67nm,100nm] is considered the largest size, and thus, it is expected to have the smallest surface area to volume ratio, and the least levels of Ag ions release inside the alveolar space. So it will be considered the least in cytotoxicity.

VI. Shape-Dependent Cytotoxicity On Alveolar Epithelial Cells:

Silver nanoparticles shapes conducted in this study are spherical and wire silver nanoparticles. A study conducted for the purpose of comparing the cytotoxicity on humans' alveolar epithelial cells caused by spherical and wire silver nanoparticles showed that wire silver nanoparticles have higher cytotoxicity on alveolar epithelial cells. Wire silver nanoparticles were marked to cause a significant reduction in cells viability and a remarkable release of LDH. However, spherical silver nanoparticles showed no toxic affect on alveolar epithelial cells. [13]. Also, wire shaped nanoparticles are well-known for their significant surface area to volume ratio [3] [9], meaning that the chance of having more chemical reactions is high. In the case of silver wire nanoparticles the increase in chemical reactions will cause more release of Ag+ which is stated to be a main cause of cytotoxicity in this research.

VII. Surface Charge-Dependent Cytotoxicity On Alveolar Epithelial Cells

In Studies conducted to clarify the effect of silver nanoparticles surface charge on bacterial cells, it has been proven that negative silver nanoparticles (which have negative zeta potential) are less toxic than positive ones. This is due to the repulsion happens between negative silver nanoparticles and the negatively charged membrane of bacteria cells, which works as an electrostatic barrier that reduces the cell-particle interaction, thus reducing the level of cytotoxicity. However, in the case of positively charged silver nanoparticles the toxicity was increased due to the attraction between them and the cells' negative membrane which triggered the incidence of chemical reactions between positive silver nanoparticles and the cells' membrane [2] [6]. Healthy human body cells were found to have an electric charge equal to -70 mv across the cell membrane [1], meaning that it is negatively charged. Thus, it can be concluded that positive silver nanoparticles have a higher level of cytotoxicity than negative ones on alveolar epithelial cells.

VIII. Proposed Work

Our proposed research, classification of inhaled silver nanoparticles cytotoxicity on alveolar epithelial cells using artificial neural network work is demonstrated here in two sections:

A. Dealing with An Integrated Set Of Parameters And Predicting Cytotoxicity

As mentioned previously, this research aims to predict the level of silver nanoparticles cytotoxicity on workers' alveolar epithelial cells using a set of chemical and physical parameters. The method conducted here depends on the variation of the three parameters in the same silver nanoparticle, where the percentage of cytotoxicity simulating parameters to none or less cytotoxicity simulating parameters is considered. For example: if the percentage is 2:1 this means there are two cytotoxicity simulating parameters and one less simulating cytotoxicity parameter. This means that the chance of having a high toxic particle is greater since the cytotoxicity simulating parameters are more. However, a percentage like 1:2 is not expected to be a low toxicity case. Rather, it is expected to be a medium toxicity case, because the one cytotoxicity simulating parameter cannot be ignored completely saying that the toxicity is low. The only case where the cytotoxicity is expected to be low is when having a percentage like 0:3 meaning that all three parameters slightly simulate the cytotoxicity. On the other hand, having the percentage of 3:0 means that all three parameters highly simulate cytotoxicity. So, such a particle is expected to have a high level of toxicity.

B. Cytotoxicity Effecting Parameters Proposed Matrix

Table I contains the rules used for controlling the process of classification, where each rule indicates a particular class of cytotoxicity. Different size ranges are given different numbers to distinguish between them, where small size is referred to as 1, medium size is referred to as 2, and large size is referred to as 3. Also,

numbers where used to distinguish between different shapes and charges. Number 2 in the case of describing the shape indicates a spherical silver nanoparticle, but number 1 indicates wire silver nanoparticles. In the case of surface charge, number 1 indicates positive silver nanoparticle, but number 2 indicates a negative one. Example: Rule (1, 1, 1) in table I indicates that the tested silver nanoparticle is small, wire shaped, and positive. In this example, the percentage of cytotoxicity simulating parameters to none or less cytotoxicity simulating parameters is 3:0, such a particle is expected to have high cytotoxicity according to the standards conducted in this research. It can be noticed from table I that the dominant class of the cytotoxicity is the high cytotoxicity. Actually, this does make sense because the prediction of the cytotoxicity levels is being conducted for workers who inhale silver nanoparticles frequently in the workplace. Notice that this classification is only among the silver nanoparticles which have the size of [1nm, 100nm], and doesn't predict the cytotoxicity of any silver nanoparticle out of this range.

IX. Modeling of the Proposed Neural Network

The classification neural network proposed in this research is a multilayer feedforward back-propagation neural network. This particular type of ANN was choosen since it fits the Complexity level of this research. Multilayer neural networks are well known for their ability to deal with complex data. Also, they are capable of learning from experience and training. Back-propagation learning algorithm was particularly choosen as training method for the ANN because it allows learning from errors, where the neural network acts like an expert after being trained on a training dataset for several times, and can deal with new dataset resulting in accurate results [20]. The proposed neural network is feedforward because there is no data have to be returned to previous layers during the classification process. The neural network is set to have the following components:

1) One input layer with three units.
2) One hidden layer with ten neurons.
3) One output layer with one neuron.
4) Two biases units.
5) Suitable set of weights.

A. The Input Layer:

This layer contains three units. The first neuron in the input layer will be given the size of the tested silver nanoparticle [1nm, 100nm]. The second neuron will be given the shape value, where 1 indicates wire shape, and 2 indicates spherical shape. The third unit will be given the surface charge value, 1 if it is positive, and 2 if it is negative.

B. The Hidden Layer:

This layer contains ten neurons. Each hidden neuron is connected to one bias. Neurons in this layer use hyperbolic tangent activation function.

$$\tanh(x)= (e^{x} – e^{x}) /(e^{x} + e^{-x}). \quad (1)$$

It has the range (-1,1) [12]. Hyperbolic activation function was choosen over other activation functions for its broad output range which is more effecient in classification performance in multilayer neural network. Also, it has been stated in the literature that using the hyperbolic tangent activation function causes fast convergence of learning algorithms.[18]

C. The Output Layer

This layer has one neuron only, which is connected to one bias. The output neuron uses linear function (identity function) as an activation function.

$$F(x)= x. \quad (2)$$

It has the range $(-\infty,\infty)$ [12]. The linear activation function was used in the output neuron due to the fact that the combination of the hyperbolic tangent and linear activation functions was found more effecient for the classification problems [18]. The output value given by the output neuron will identify the class under which the tested silver nanoparticle is classified, where the value of 0 refers to low toxicity class, .5 refers to medium toxicity class, and 1 indicates high toxicity class. Getting an output which is silghtly different from the stated output values is not a problem where it can be rounded to match the closest value to it. For example, -.0517 is close to 0 , so such an output reflects a low toxicity class.

X. Experement

A programming code was written to verify the proposed network ability to classify the cytotoxicity of inhaled nanoparticles using Matlab program.

A. Training Stage

This classification feedforward neural network was trained using back-propagation learning algorithm. Dataset used cosists of 100 random different points. Training epochs (iterations) were 100 epochs, see Fig 1. Dataset was picked randomly for the purpose of avoiding biasness and ensuring the reliability of the proposed ANN. The whole dataset was used as training dataset. In order to avoid overfitting Bayesian Regularization technique was used. After 100 training epoches the neural network was able to reach its lowest error value which is .0014 see Fig 2. The neural network training regression was equal to .99593 which reflects almost a perfect relationship between the target values and the output of the neural network. See Fig 3.

B. Testing Stage:

The proposed neural network was tested on different 400 points . See testing data plot in Fig 4. The output of the testing data has a minimum value equal to -.0517 which is very close to zero, meaning that it indicates low toxicity, and a maximum value equal to 1.0742 which is very close to 1 (high toxicity). See Fig 5.

C. Results:

The proposed neural network showed high effeciency in classifying inhaled silver nanoparticles cytotoxicity on human's alveolar epithelial cells. The results of the neural network matched the parameters matrix proposed in this research (table I), where the dominant class was high toxicity class, followed by medium toxicity class, to end up with low toxicity class which is the least dominant class, see Fig 6.

XI. Conclusion

The usage of silver based nanoparticles in different industries is expanding due to their antibacterial effect. However, many studies prove that silver nanoparticles can interact differently with human's body causing different levels of damage. Inhalation is the most common way by which we are exposed to silver nanoparticles specially those who work in such a field. In order to prevent health risks we have to assess the potential cytotoxicity of engineered silver nanoparticles before releasing them into industries. This research shows that silver nanoparticles cytotoxicity on workers' alveolar epithelial cells can be assessed in a better way if we use integrated set of parameters rather than using one parameter only. The artificial neural network has been used to predict the level of inhaled silver nanoparticles cytotoxicity. The proposed neural network showed a high level of reliability and efficiency in classifying the cytotoxicity of inhaled silver nanoparticles.

XII. Future Work

This research is closely related to human health. So, it will be an attractive area for future research. The number of parameters used in the classification process can be increased in the future in accordance to the need**.** Also, this neural network can be used to predict the cytotoxicity level of new engineered silver nanoparticles.

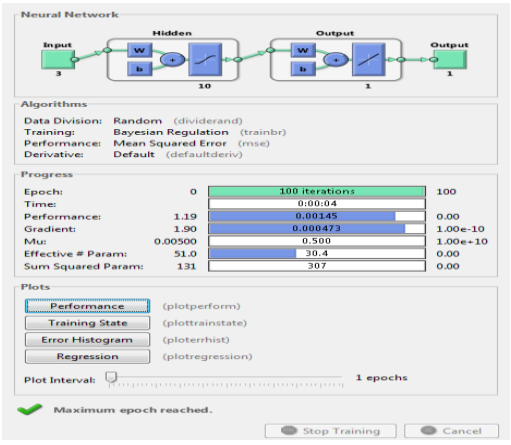

Figure 1. ANN options showing 100 training epochs.

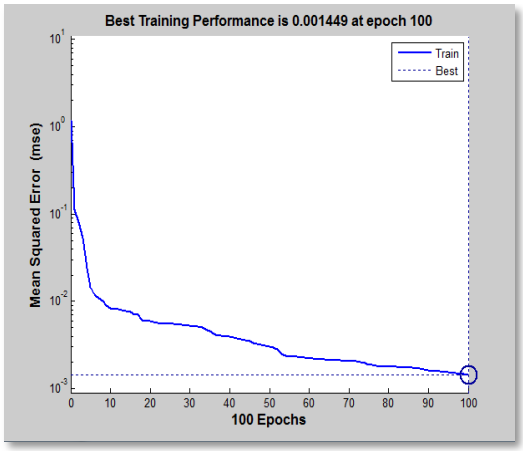

Figure 2. Error decrease during 100 training epochs.

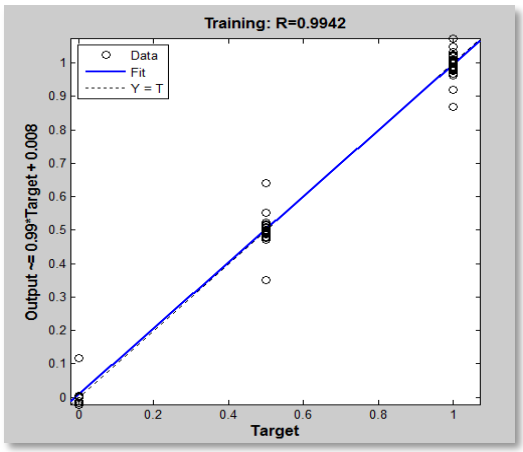

Figure 3. Target values VS. ANN output.

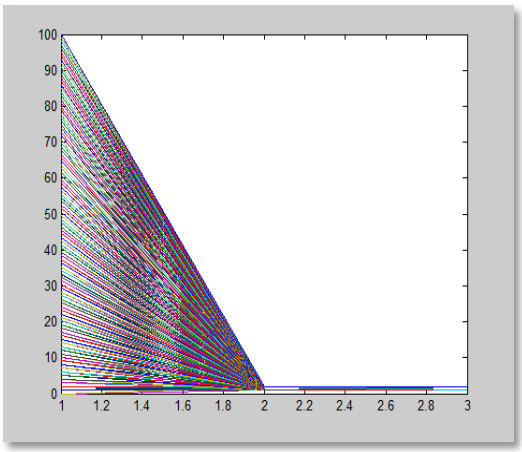

Figure 4. Testing data.

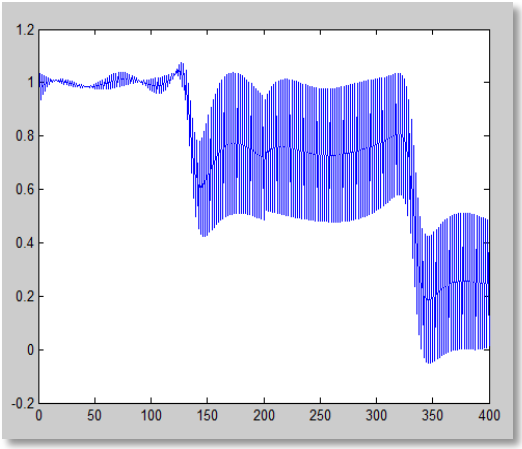

Figure 5. Testing data output.

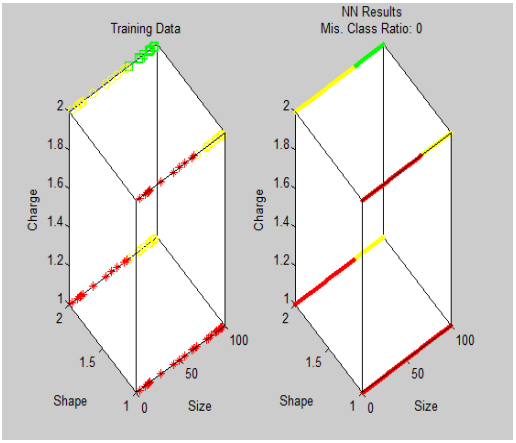

Figure 6. ANN results.

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TABLE I.
RULES USED FOR CONTROLLING THE PROCESS OF CLASSIFICATION.

Size	Small (1) [1nm,33.33nm]	High Tox. Rule (1,1,1)	High Tox. Rule (1,1,2)	High Tox. Rule (1,2,1)	*Med. Tox. Rule (1,2,2)*
	Medium (2) (33.33nm,66.67nm)	High Tox. Rule (2,1,1)	High Tox. Rule (2,1,2)	High. Tox. Rule (2,2,1)	*Med. Tox. Rule(2,2,2)*
	Large (3) (66.67nm,100nm]	High Tox. Rule (3,1,1)	*Med. Tox Rule (3,1,2)*	*Med.Tox. Rule(3,2,1)*	*Low Tox. Rule (3,2,2)*
		Wires (1)	Sperical (2)	Positive (1)	Negative (2)
		Shape		Surface Charge	