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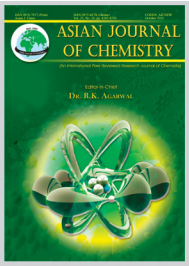

Study on Toxicity of Seven Manufactured Nano Particles and Two Phenolic Compounds to Bacteria *Vibrio fischeri* Using Homemade Luminometer

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Acute toxicity of nano SiO_2, ZnO, MCM-41 (Meso pore silica), Cu, multi-walled carbon nanotube, single walled carbon nanotube, Fe (coated) and two phenol derivatives (hydroxyphenol and 4-aminophenol) to bacteria *Vibrio fischeri* by bioluminescence inhibition test, using a homemade luminometer, was evaluated. The values of the nominal effective concentrations, causing 20 % and 50 % inhibition of bioluminescence, using two mathematical models, Weibull and gamma at two times of 5 and 30 min were calculated. The results of each model were compared using linear regression. Luminometer was designed with photomultiplier detector. Luminol chemiluminescence reaction was carried out for the calibration graph. In the linear calibration range, the correlation coefficients and coefficient of variation were 0.988 and 3.21 % respectively, which demonstrate the accuracy and reproducibility of the instrument that are suitable. The important part of this research depends on how to optimize the best condition for maximum bioluminescence. The culture of *Vibrio fischeri* with optimal conditions in liquid media, were stirring at 120 rpm at a temperature of 23 to 26 ºC and were incubated for 24 to 48 h while solid medium was held at 18 ºC and for 48 h. Suspension of nanoparticles ZnO, after 30 min contact time to bacteria *Vibrio fischeri*, showed the highest toxicity while SiO_2 nanoparticles showed the lowest toxicity. After 5 min exposure time, the toxicity of ZnO was the strongest and MCM-41 was the weakest toxicant component. As can be known, this is the first investigation that evaluates the toxicity of nano materials SiO_2, MCM-41, Fe (Coated), multi-walled carbon nanotube, single walled carbon nanotube and phenolic components (hydroxyphenol and 4-aminophenol) to *Vibrio fischeri* by two mathematical models.

Key Words: *Vibrio fischeri*, Effective concentration, Toxicity, Nanomaterials, Bioluminescence.

INTRODUCTION

The applications of nanotechnology in the form of nanomaterials have increased, so research on the effects of exposure to nanomaterials and their toxicity is very important, especially the domestic and industrial wastewater is likely to spread there. Diagnostic tests for toxicity in recent years has grown continuously and they are useful tools for evaluating the spread of toxic substances into the environment. A lot of analytical methods that are used to check the pollution of the environment, require expensive equipments and sampling from environment which are time consuming. These problems are solved by one of these methods using the bacteria, as a bio-sensor. This method is sensitive (responsing to the very low concentrations of particles), low-cost and easily reproducible and takes 5 to 30 min to predict toxicity. *Vibrio fischeri* is a luminescent bacteria can be used in a toxicity test. The use of *Vibrio fischeri* bacteria in the bioluminescence inhibition test has the advantages that mentioned above, also sometimes, it can solve the ethical problems arising from the use of animals (fish, mice, *etc.*). A biosensor is an analytical device that combines a biological sensing element with a transducer to produce a signal proportional to the analyte concentration[1]. Biosensors have been extensively applied in clinical, food and environmental areas due to the advantages of fast detection speed, high selectivity and sensitivity[2]. Risk hazards of nano particles are different because they do not behave as predicted. Nanoparticles offer unique, physical, chemical, electrical and optical properties while are generating toxins, cancer and allergies[3]. The main mechanism of toxicity of nanoparticles resulting from oxidation stress (OS) that lipids, carbohydrates, proteins and DNA damage[4]. ZnO and CuO nanoparticles are used as an antibacterial protection in dentistry and as the construction of wood and antibacterial cloth, respetively[5,6]. Preparation of ZnO nanoparticles used in cosmetics and sunscreens is increasing day by day, because they reflect ultra-violet better than larger particles[7]. The preparation of nanoparticles for self-cleaning coating are also used[8]. Nano

ZnO, in preparing the catalysts, ceramics and coloured materials is used. Silica nano particles (silicon dioxide SiO_2) have importance in fabrication of catalyst support, electrical and thermal insulators and also are used in the coating process, creating molecular sieve adsorbents and filler materials[9]. In medicine and pharmacy are used as drug carriers[10] and also for gene delivery[11]. Mesoporous silicates, such as MCM-41 (the most common mesoporous silicates), are porous silicates with huge surface areas (normally ≥ 1000 m^2/g), large pore sizes (2 nm ≤ size ≤ 20 nm) and ordered arrays of cylindrical mesopores with very regular pore morphology.

The large surface areas of these solids increase the probability that a reactant molecule in solution will come into contact with the catalyst surface and react. The large pore size and ordered pore morphology allow one to be sure that the reactant molecules are small enough to diffuse into the pores. Today, mesoporous silica nanoparticles have many applications in catalysis, drug delivery and imaging[12]. A study was conducted in 2009 by Kasemets *et al.*[13]. On toxic effects of nanoparticles ZnO, Cuo and TiO_2 on the single-cell eukaryotic organisms *Saccharomyces cerevisiae*. The effect of metal oxide nanoparticles, the bulk and ion formation were compared. Both formulation of ZnO showed the same toxicity. Nano CuO was 60 times more toxic than bulk CuO. The reason of increase of toxicity (nano and bulk CuO) after 24 h exposure time than 8 h, was increase of copper ion dissolution in excess times.

The study was conducted by Zhang *et al*.[14] for nano ZnO, the mechanisms of membrane destruction and oxidation stress as an antibacterial agents on the bacteria *Escherichia coli*. Toxicity of nanoparticles and bulk ZnO, TiO_2, CuO to bacteria *Vibrio fischeri*, *Daphnia magna* and *Thamnocephalus platyurus* species of crustaceans, were analyzed by Heinlaan *et al*.[15]. ZnO components (nano and bulk) and $ZnSO_4$ on three species were highly toxic. Unlike zinc and copper compounds on three species showed different toxity provided copper ions were more toxic than bulk and nano copper oxide. Jiang *et al*.[16] investigated on toxicity of nanoparticles SiO_2, Al_2O_3, TiO_2 and ZnO to bacteria *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens* and compared the results with the toxicity of compounds in bulk formulation. All the nano particles except TiO_2 were more toxic than their bulk formulation. ZnO nano particles were more toxic than three other nano particles and 100 % of the bacteria were destroyed. SiO_2 nano particles killed 40 % *B. subtilis*, 58 % *E. coli* and 70 % *P. fluorescens*. Flash assay which is performed in microplate can be used as a method with high efficiency, low cost and quick to measure the toxicity of nano particles (antibacterial properties) to the bacteria *Vibrio fischeri*. In the study, 11 materials with different properties in two groups of particles (metallic and organic) and metal salts in cuvette and microplate in flash assay were examined. EC_{50} values after 30 min exposure time to nano scale organic cationic polymers, were between 215 to 775 mg/L. for metal oxides, EC_{50} values after 30 min,were about 4,100 and 4000 mg/L for ZnO (bulk and nano formulation), nano CuO and bulk CuO, respectively[17]. Size dependent properties of nano materials such as difference in toxicity have been proved. Therefore, particular properties of nano materials (large specific area) may produce different biological effects than materials in micro size. Arthritis, tuberculosis and chronic renal disease are the phenomenon of contact to micro sized silica[18,19]. Cytotoxicity effect of SiO_2 nano particles (15 and 46 nm) in human bronchoalveolar carcinoma-derived cells and the oxidative stress mechanisms, which is caused by nano silica (15 nm) was evaluated by Lin *et al.*[20]. Silica nano particles with different concentrations were dispersed in the medium by changing the concentrations (10 to 100 mg mL) and exposure time (24, 48 and 72 h) of both size of silica, cell viability decreased. Carbon nanotubes have thermal, chemical, electrical and mechanical properties uniquely. Reforms and chemical changes in carbon nanotubes for solubility in water, length, dimater, aspect ratio of nanotubes, their type and impurities are the effective parameters of the nanotubes that produce toxicity. In a research, suspensions of carbon nanotubes (single walled and multi walled) and C60 were prepared by sonication process. Then they were examined by UV radiation in order to inactivate the bacteria *Vibrio fischeri* and production of reactive oxygen species (ROS)[21]. This study showed that reactive oxygen species production and increase of toxicity are indicating the size reduction and contrary. Zhu *et al*.[22] sudied on acute toxicity of single walled carbon nanotube and multi-walled carbon nanotube to species *Daphnia magna* after 48 h exposure time. This assessment was based on inactivity and mortality as end points of toxicology. This research exhibited that single walled carbon nanotube was more toxic than multi-walled carbon nanotube. Cytotoxicity of macrophage after 6 h contact time with both types of carbon nanotubes was investigated[23]. This study showed that the biological activity of cells in the vicinity of both types of nanotubes reduced and phagocytic activity was destroyed. Toxicity studies showed that the cytotoxicity of single walled carbon nanotubes were more than multi-walled carbon nanotubes. Applications of copper nano particles are in the manufacture of ceramics, films, polymers, oils, lubricants, coatings and electronic components[24]. The copper nano particles are used in pharmaceutical and as antibacterial substances[25]. Toxicity of copper nano particles to *E. coli* and *Bacillus subtilis* using the agar plate test, were studied by Yoon *et al*.[26]. The results demonstrated that antimicrobial properties of copper nano particles to both types of bacteria. The toxicity of nano particles copper/sepiolite on *E. coli* and *S. aureus* were evaluated and was observed that the growth of bacteria was limited to 99/99 % by nano particles[27]. Rispoli *et al*.[28] studied about the effects of aeration, concentration of nano particles, pH, concentration of bacteria and temperature on toxicity of nano copper based on *E. coli* test. The iron nano particles are used in biological separation and detection of biological (cells, proteins, bacteria, viruses, enzymes, nucleic acid), clinical diagnoses [MRI (magnetic resonance image)] and drug delivery[29]. Unfortunately, there were no appropriate studies on toxicity of nano iron and nano iron oxide. Phenolic compounds in the aquatic environment can be produced from industries such as pulp industries, agricultural and petrochemical activities. Fluid from washing the solid waste in production of oil from oil-shale include the phenolic compounds that are phenol, hydroxyphenols, aminophenols, 2,4-dichlorophenol and poly-chlorine[30,31]. In 2006, recombinant bactrial sensors identifying phenolic compounds were built by Leedjarv *et al.*[32]. The sensor bacteria *Pseudomonas fluorescens* OS8

(pDNdmpRlux) were inducible by phenol, cresols, 2,3-, 2,4-, 2,6- and 3,4-dimethylphenol, resorcinol and 5-methylresorcinol. The detection limits for phenol compounds were 0.03, 42.7 and 0.08 mg/L for 2-methylphenol, 5-methylresorcinol and phenol, respectively that were the most plentiful phenolic contaminant in the surroundings. Various phenolic compounds showed an additive influence on the inducibility of the snsor. Many studies about the toxic nature of nanoparticles were performed on animals and plants, but studies on micro organisms, especially bacteria *Vibrio fischeri* are limited.

In the this study, we calculated the effective concentration (EC) of nano materials and two phenolic compounds (as standard toxin) causing 20 % and 50 % inhibition of bioluminescence to *Vibrio fischeri* using the homemade luminometer by two models: The gamma model and the Weibull distribution model, also the optimized conditions of culture for maximum bioluminescence of *V. fischeri* were determined. This is the first toxicity evaluation of nano Fe (coated), SiO_2, MCM-41, single walled carbon nanotube, multi-walled carbon nanotube, Cu, hydroxyphenol and 4-amiophenol toxicity to *V. fischeri* by two mathematical models.

EXPERIMENTAL

All nanosized materials were purchased from Nanotechnology Research Center, Research Institute of Petrolum Industry, Iran (RIPI) with particle sizes of 10-50 nm for nano ZnO, 60-100 nm for SiO_2, 60-150 nm for MCM-41, 10-20 nm diameter and mean 10 μm length for multi-walled carbon nanotube, 2-3 nm diameter and mean 10 μm length for single walled carbon nanotube and 5-25 nm for coated nano iron. The purity of materials were 95 % for both type of carbon nanotubes, 24 % for nano iron and 99.5 % for other nano particles. Sodium dodecyl sulphate (SDS), luminol, $CuSO_4.5H_2O$, hydroxyphenol [$C_6H_4(OH)_2$], 4-aminophenol ($C_6H_4OHNH_2$) and hydrogen peroxide (H_2O_2 35 %) were purchased from Fulka Chemical Company (Buchs, Switzerland). The sodium dodecyl sulphate solution in its critical micelle concentration (CMC) was prepared (7-10 mM) and the stock suspensions of nano materials in sodium dodecyl sulphate were sonicated for 30 min and stored in dimness at refrigerator. Hydroxyphenol and 4-aminophenol were dissolved in deionized water and were not sonicated. The stock concentrations were 6000, 6000, 6000, 2000, 1200, 300, 300, 300, 300 ppm for nano Fe (coated), SiO_2, MCM-41, 4-aminophenol, Cu, hydroxyphenol, multi-walled carbon nanotube, ZnO, single walled carbon nanotube respectively.before toxicity experiments, stockes were wortexed.

Organism and nutrient media: *Vibrio fischeri* strain DSM 507 was obtained from Iranian Research Organization for Science and Technology (IROST). To ensure the best quality of luminescent bacteria with maintainable viability, the bacteria can be inoculated and maintained in culture medium. Although different cultures can be used, the following cultures medium allow greatest luminescence, growth and solidity that are practical for the mentioned procedure in this way, three basic growth media were examined: (1) Bacto marine broth (DIFCO 2216) (Table-1); (2) Sea water agar (twin pack) (Table-2); (3) Sea water agar (Table-3).

TABLE-1
NUTRIENT MEDIA FOR REVIVING BACTERIA [DSMZ MEDIUM 514: BACTOMARINE BROTH (Difco 2216)]

Bacto marine broth (Difco 2216)	Amounts (g)
Bacto peptone	5.0
Bacto yeast extract	1.0
Fe (III) citrate	0.1
NaCl	19.4
$MgCl_2$ (dried)	5.9
Na_2SO_4	3.2
$CaCl_2$	1.8
KCl	0.5
Na_2CO_3	0.1
$SrCl_2$	34.0
H_3BO_3	22.0
Na-silicate	4.0
NaF	2.4
(NH_4) NO_3	1.6
Na_2HPO_4	8.0
Distilled water (mL)	1000

TABLE-2
NUTRIENT MEDIA FOR SOLID CULTURES
SEA WATER AGAR (TWIN PACK)

Part A	Standard formula (g L^{-1})
	5
Yeast extract	5
Beef extract	3
Agar	15
Part B	**Standard formula (g L^{-1})**
NaCl	24.0
KCl	0.7
$MgCl_2$	5.3
$MgSO_4 \cdot 7H_2O$	7.0
$CaCl_2$	0.1

TABLE-3
NUTRIENT MEDIA FOR LIQUID CULTURES
(DSMZ MEDIUM 246: SEA WATER AGAR)

Media	Amounts
Sea water agar	
Beef extract	10 g
Peptone	10 g
Agar	20 g
Tap water	250 mL
Sea water*	750 mL
*Artificial sea water	
NaCl	28.13 g
KCl	0.77 g
$CaCl_2 \cdot 2H_2O$	1.60 g
$MgCl_2 \cdot 6H_2O$	4.80 g
$NaHCO_3$	0.11 g
$MgSO_4 \cdot 7H_2O$	3.50 g
Distilled water	1000 mL

The first media was used for reviving; the second one was used for solid cultures and the third one for liquid cultures. The bioluminescence of *Vibrio fischeri* in sea water agar culture (solid media) has been shown in Fig. 1.

Solid cultures were retained in incubator at 18 ºC. After inoculation with luminous *V. fischeri* from solid culture, liquid cultures were incubated for 48 h at 25 ºC in an orbital shaker at 120 rpm[33].

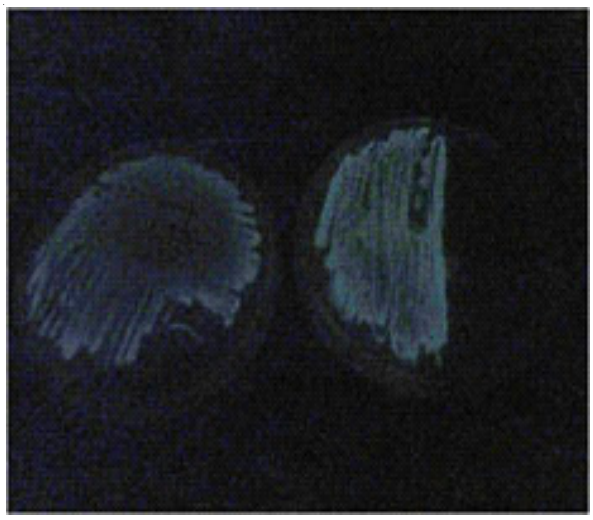

Fig. 1. Bioluminescence of bacteria *Vibrio fischeri* in solid culture

Assay procedure and data analysis: Flash assay is a test that inhibits *V. fischeri* luminescence and was done by homemade luminometer that will be illustrated in the following section. A 1.5 mL volume of bacterial suspension was contacted with 0.5 mL volume of suspension of nano materials. All suspensions and phenolic components were diluted in sodium dodecyl sulphate solution and deionized water, respectively. The decrease in bacterial luminescence (INH %) due to addition of test samples was calculated as follows:

$$INH\% = 100 - \frac{IT_T}{IT_0 \times KF} \times 100 \text{ with } KF = \frac{IC_T}{IC_0}$$

where, KF is the correction factor based on control, IC_0 and IT_0 are the initial luminescene intensities of control and test samples. IC_T and IT_T are the luminescence intensities of the control and test samples after T min contact time. EC_{50} and EC_{20} values are the concentrations of toxicants (mg/L) causing 50 % and 20 % decrease in bioluminescence after T min exposure time, respectively. Up to now, various exposure times have been used, *e.g.*, 5, 15, 30 and 45 min in this study, 5 min and 30 min were seclected as operational times. Three independent assays were carried out. The data for percentage inhibition obtained in each experiment were converted to γ values according to first model, where:

$$\gamma = \% \text{ inhibition}/(100 - \% \text{ inhibition})$$

γ values were plotted against their corresponding chemical concentrations, after first converting all data to natural logs (Ln), to generate Ln γ/Ln concentration curves for each chemical in this model. Values falling within the 10-90 % inhibition range were used to fit a straight line to the Ln-transformed data by linear regression and the resulting equations used to calculate the EC_{20} and EC_{50} for each compound, the EC_{20} and EC_{50} values have been calculated from linear regression equations of dose/response curves of the form:

$$Ln\ y = mLn\ x + c$$

where, Ln is the natural log, y is the value for γ, x is the dose (ppm), m and c are the slope and intercept, respectively. In the second model, the concentration and response (INH) obtained from each experiment, are fitted by two parametric Weibull distribution equation:

$$F(C) = [1\text{-}exp(\text{-}exp(k_1 + k_2 log_{10}(C)]$$

In this equation, F(C) represents the amounts of INH, C is the concentration (mg/L), k_1 and k_2 are the location and the slope parameters, repectively. With linear regression of Weibull equation, the plotting ln[-ln(1-F(C)] values as the y-axis *versus* log_{10}(C) values as the axis of x, k_1 and k_2 are calculated. With an equation as follows, EC_{20} and EC_{50} values are calculated with the INH = 0.2 and INH = 0.5.

$$y = k_1 + k_2 x$$

where x, y are log_{10}(C) and ln(-ln(1-F(C))), respectively.

Bioluminescence detection was carried out by a homemade luminometer supplied with a model R-446 photomultiplier (PMT) (Hamamatsu, Japan). The luminometer connected to a personal computer *via* a suitable interface (Micropars, Tehran, Iran) as shown in Fig. 2. experiments were done in double layer cuvettes of 49 mm^2 internal cross sectional area, 100 mm^2 external cross sectional area and 45 mm altitude at 25 ºC. Bioluminescence intensity was recorded as function of time, the time resolution of the luminometer was 0.01s.

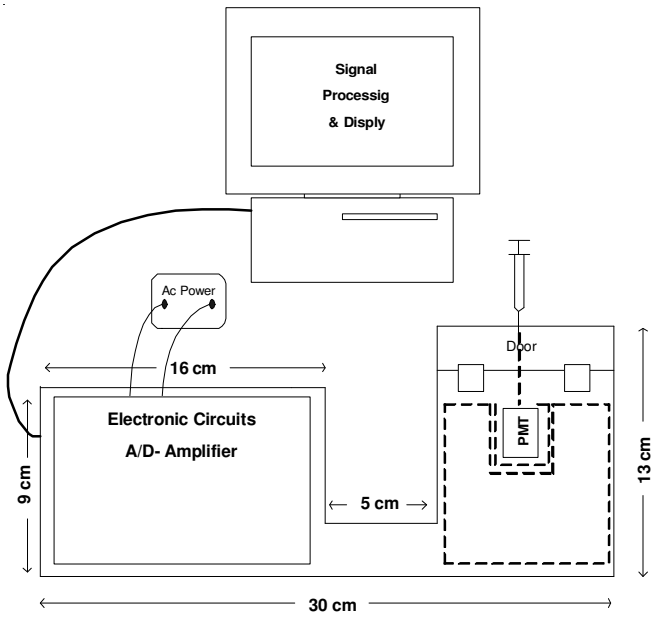

Fig. 2. Schematic representation of homemade luminometer for measuring bioluminescence

Calibration of device was performed by the luminol luminescence reaction[34]. The luminol luminescence reaction is one of the most effective non-biological system producing light or chemiluminescence (Fig. 3). For evaluating the precision, accuracy, reproducibility of device, some quantities should be calculated as follows[35]. For calculating the quantitis, the results of mentioned reaction have been used.

$$\text{Limit of detection (LOD)} = \frac{3S_a}{\beta}$$

$$\text{Limit of quantity (LOQ)} = \frac{10S_a}{\beta}$$

Limit of linear range (LLR)

Linear dynamic range (LDR), which is difference between LLR and LOQ

Coefficient of variation (CV) = $\frac{S_d}{\bar{x}}$

where, S_a, S_d and $\bar{x}$ are standard deviation of intercept, standard deviation of luminol concentrations (triple tests at three different luminol concentrations in linear range of calibration diagram were carried out) and average of three luminal concentrations in each test, respectively. S_a was calculated as follows:

$$S_\alpha = S_r\sqrt{\frac{\sum x_i^2}{n.S_{xx}}},\ S_r = \sqrt{\frac{S_{yy} - \beta^2 S_{xx}}{n-2}},\ S_{xx} = \sum x_i^2 - \frac{(\sum x_i)^2}{n},$$

$$S_{yy} = \sum y_i^2 - \frac{(\sum y_i)^2}{n},\ \beta = \frac{S_{xy}}{S_{xx}},\ S_{xy} = \sum x_i y_i - \frac{\sum x_i \sum y_i}{n}$$

In these equations n, y, x, β are number of luminol concentrations in linear range, intensity of chemiluminescence, luminol concentration and slope of calibration line, respectively.

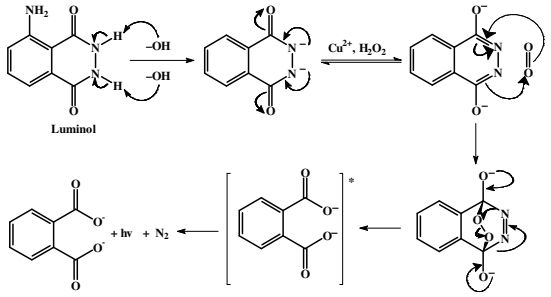

Fig. 3. Mechanism of luminol chemiluminescence reaction

In Table-4, The quantities LOD, LOQ, LLR, LDR, CV are given. In Fig. 4, in the linear range of concentrations and responses, the quantities LLR, LDR are visible.

TABLE-4
FIGURE OF MERIT (PRECISENESS, ACCURACY AND REPEATABILITY) OF CHEMILUMINESCENCE TEST

Quantities	Amounts
Limit of detection	0.16 ppm
Limit of quantity	0.53 ppm
Limit of dynamic range	2.47 ppm
Limit of linear range	3.00 ppm
Coefficient variation	3.21 %

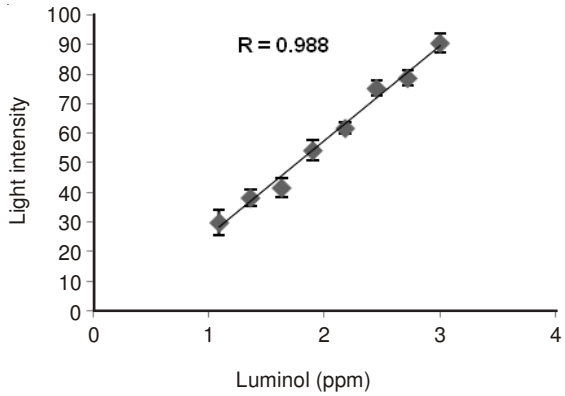

Fig. 4. Correlation diagram (calibration diagram) for Chemiluminescence emission intensity as a function of luminol concentration. The all reagent concentratioins are: $CuSO_4$ (6×10^{-3} M, 0.1 mL), hydrogen peroxide (10 %, 0.1 mL), water (1 mL) and varying concentrations of luminal solution in NaOH (0.1 M) : (1) 1.09 ppm, (2) 1.36 ppm, (3) 1.63 ppm, (4) 1.9 ppm, (5) 2.18 ppm, (6) 2.45 ppm, (7) 2.72 ppm, (8) 3 ppm

RESULTS AND DISCUSSION

Toxicity of nano particles SiO_2, ZnO, MCM-41 (Meso pore silica), Cu, multi-walled carbon nanotube, single walled carbon nanotube and Fe (Coated) and two derivatives of phenol (hydroxyphenol and 4-amino phenol) to bacteria *Vibrio fischeri* were evaluated. The values of EC_{50} and EC_{20} at 5 min and 30 min contact times were calculated using two mathematical models. At 30 min contact time, the suspension of SiO_2 showed the lowest toxicity, means EC_{50} values calculated from two models for SiO_2 were greater than that of the other reagents. In contrast, the suspensions of ZnO showed the highest toxicity (Table-5). EC_{50} values obtained for ZnO from two models are comparable with effective concentrations of ZnO suspensions obtained by Heinllan *et al.*[15] (1.9 ppm) and Mortimer *et al.*[17] (4.8 ppm). The test results and calculation of effective concentration (EC) values showed that MCM-41 and nano ZnO have the lowest and highest toxicity after 5 min exposure time to *V. fischeri* respectively (Table-6). Hydroxyphenol was more toxic than 4-aminophenol, probably due to the existence of two hydroxy groups (OH) in its structure. As shown in Table-5, EC_{50} values calculated for the hydroxyphenol by both models, are closer to EC_{50} values of nano ZnO and CNTs that show the hydroxyphenol in terms of toxicity is in the second level. But after 5 min exposure time, according to EC values calculated by two models,both CNTs were more toxic than phenolic compounds. The reason of observation is probably belong to the rapid diffusion of CNTs into *V. fischeri* and damaging of the cell at short time. Mortimer *et al.*[17] reported the EC_{50} values of 3,5-dichlorophenol after 30 sec and 30 min contact time to *V. fischeri*, in about 6.2 ppm and 3.1 ppm, respectively. EC_{50} values calculated by both models for hydroxyphenol after 5 min (Table-6) are very similar to EC_{50} values calculated by Jennings *et al.*[36] for the phenols with three systems including Toxalert (42.5 ppm), Microtox (34.8 ppm) and Lumistox (30.3 ppm). MCM-41 after 30 min contact time to *Vibrio fischeri*, was more toxic than nano SiO_2. MCM-41s are listed to the latticed silica nano particles, which are quite porous and have meso pore structure, while silica (SiO_2) is nonporous-spherical nanoparticle. Single walled carbon nanotube was a little more toxic than multi-walled carbon nanotube after 30 min contact time. The reason of this matter is that single walled carbon nanotube is smaller than multi-walled carbon nanotube[21]. EC_{50} values for single walled carbon nanotube calculated by two models to *Vibro fischeri* (Table-5), are similar to the values obtained by Roberts *et al.*[37] (20 ppm to 100 % mortality), the species was *Daphnia magna*. Also, Petersen *et al.*[38] for 51 % mortality of the *Lumbriculus variegates*, reported EC_{50} value about 10 ppm. For nano copper, effective concentrations after 5 min, were not calculable, but EC_{50} values obtained after 30 min (Table-5) are comparable with the values obtained for the nano-CuO by Heinllan *et al.*[15] (EC_{50} = 79 ppm)and Mortimer *et al.*[17] (EC_{50} = 68.1 ppm). It can be concluded that copper oxide nano particles are a little more toxic than copper nano particles. The comparison of EC_{20} and EC_{50} values calculated by two models after 30 min and 5 min contact times to *V. fischeri* have been shown in Fig. 5 and 6. The required effective concentrations causing 20 % and 50 % light reduction after 30 min are less than that of required effective concentrations after 5 min. As shown in

TABLE-5
EFFECTIVE CONCENTRATION FOR CHEMICALS IN 20 % AND 50 % INHIBITION IN TWO MODELS. THE EC_{50} AND EC_{20} VALUES (ppm) WERE OBTAINED AFTER 30 MIN EXPOSURE TIME AT 25 ºC FROM GAMMA AND WEIBULL EQUATIONS

Component	Gamma model					Weibull model				
	EC_{50}	EC_{20}	m	c	r	EC_{50}	EC_{20}	K_1	K_2	r
4-Aminophenol	105.9	36.21	1.27	-5.96	0.95	127.445	40.205	-5.09	2.24	0.987
Fe(coated)	238.19	51.06	0.903	-4.94	0.848	255.45	25.05	-3.07	1.12	0.9
Hydroxyphenol	11.57	3.92	1.28	-3.13	0.934	10.945	2.18	-2.04	1.61	0.968
MCM-41	319.68	26.8	0.55	-3.25	0.963	366.855	25.38	-2.84	0.96	0.971
Cu	86.31	12.64	0.723	-3.21	0.936	89.285	12.74	-2.97	1.34	0.95
SiO_2	333.82	26.98	0.55	-3.2	0.924	381.274	27.27	-2.91	0.98	0.884
ZnO	10.24	2.14	0.88	-2.06	0.881	7.79	3.32	-3.107	3.08	0.904
MWCNT	13.87	1.52	0.62	-1.65	0.95	13.985	2.04	-2.19	1.6	0.94
SWCNT	12.13	3.73	1.17	-2.93	0.931	11.0315	3.16	-2.54	2.087	0.972

TABLE-6
EFFECTIVE CONCENTRATION FOR CHEMICALS IN 20 % AND 50 % INHIBITION IN TWO MODELS. THE EC_{50} AND EC_{20} VALUES (ppm) WERE OBTAINED AFTER 5 MIN EXPOSURE TIME AT 25 ºC m GAMMA AND WEIBULL EQUATIONS

Component	Gamma model					Weibull model				
	EC_{50}	EC_{20}	m	c	r	EC_{50}	EC_{20}	K_1	K_2	r
4-Aminophenol	200.83	84.97	1.61	-8.57	0.977	245.845	92.405	-6.74	2.66	0.972
Fe(coated)	545.42	105.15	0.84	-5.32	0.807	653.75	76.885	-3.8	1.22	0.881
Hydroxyphenol	43.9	17.29	1.5	-5.65	0.953	47.52	17.685	-4.8	2.65	0.948
MCM-41	966.5	142.95	0.72	-4.98	0.955	959.37	157.75	-4.67	1.44	0.942
Cu	Nd[a]	nd	-	-	-	nd	nd	-	-	-
SiO_2	664.15	33.03	0.46	-3	0.982	702.704	32.74	-2.78	0.85	0.97
ZnO	18.32	3.16	0.79	-2.29	0.945	16.44	2.87	-2.19	1.5	0.925
MWCNT	20.16	4.32	0.9	-2.7	0.972	18.43	5.18	-3.43	2.42	0.941
SWCNT	21.51	5.21	1.02	-3.13	0.901	17.13	4.37	-2.72	1.9	0.921

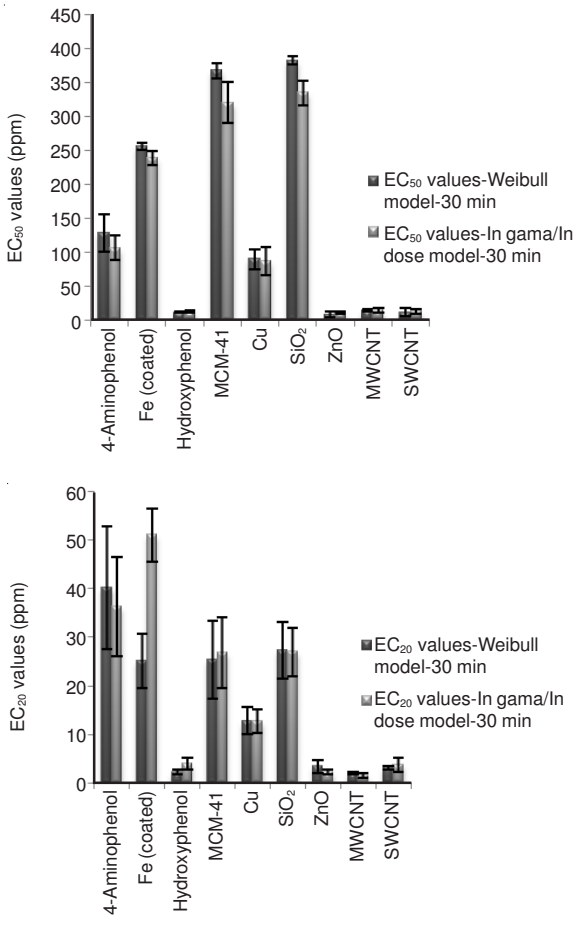

Fig. 5. Comparison of EC_{50} and EC_{20} values calculated by two models after 30 min contact time

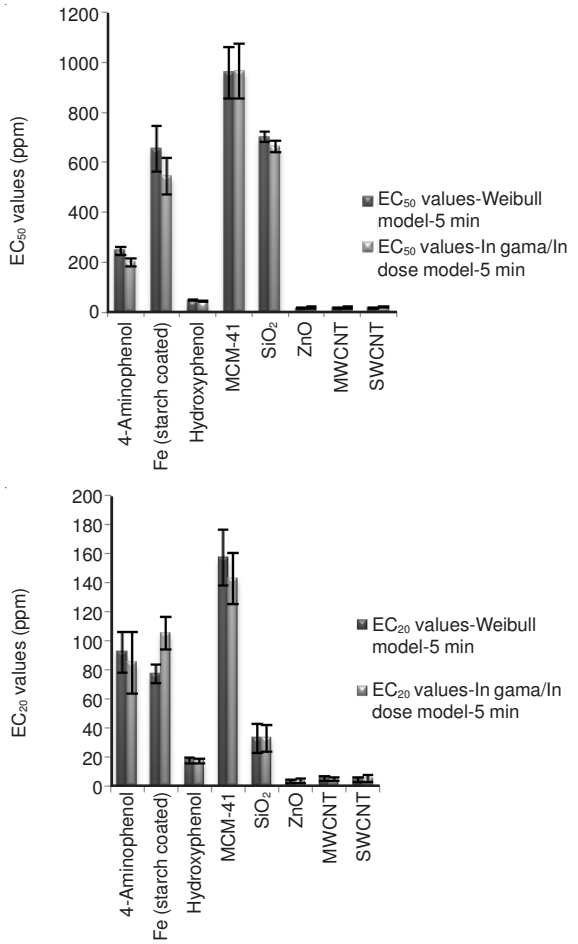

Fig. 6. Comparison of EC_{50} and EC_{20} values calculated by two models after 5 min contact time

Figs. 5 and 6 and Tables 5 and 6 concentrations of reagents in contact to *V. fischeri*, destroy more bacteria after 30 min compared to 5 min and lead to higher light inhibition (INH %) and higher toxicity. There is need enough time to diffuse to cells and ruining lipids, carbohydrates, proteins and DNA. A comparison of the EC_{20} and EC_{50} values obtained for each reagent in each model revealed great agreement with correlation coefficient (r) of between 0.754 and 0.998 (Figs. 7 and 8). There are a little differences between the regression lines for EC_{20} nad EC_{50} within two models for each contact times in a perfect experiment, the slopes of Weibull and gamma models will correspond to 1. The slope < 1 shows the experiment is not fast sensitive to exchanges in material concentration while the slope > 1 displays great changes in inhibition percentage of bioluminescence for a little changes in concentration. The evaluated slopes for all components in both models for 5 and 30 min contact times altered from < 0.5 to almost 3 (Fig. 9). As it has been shown in Fig. 9, toxicants with weak slopes lean to act in a similar manner in both models with the best consent happening when slopes were about 1. Reversely, where components gave a great response curve in one model, in another model the slope of the response was less likely to be in excellent consent. The best correlation (r = 0.78) was for 5 min contact time between two models and the lower correlation (r = 0.71) was for 30 min contact time between Weibull and gamma models.

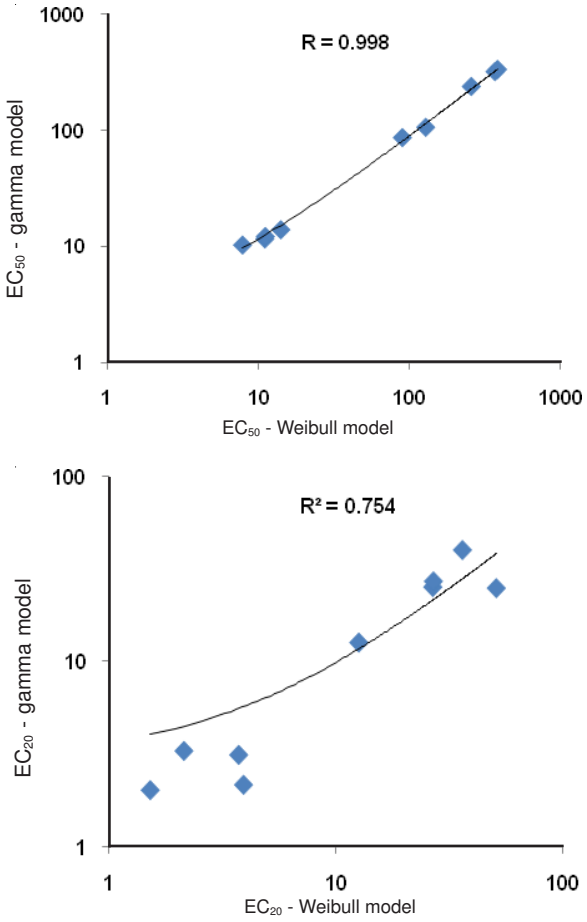

Fig. 7. Comparison of EC_{50} and EC_{20} values (ppm) obtained by two models after 30 min exposure time at 25 ºC by linear regression

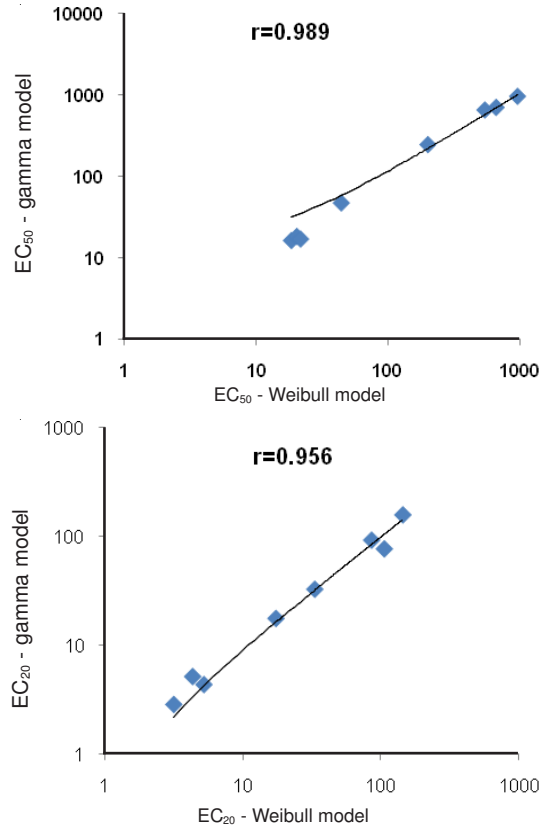

Fig. 8. Comparison of EC_{50} and EC_{20} values (ppm) obtained by two models after 5 min exposure time at 25 ºC by linear regression

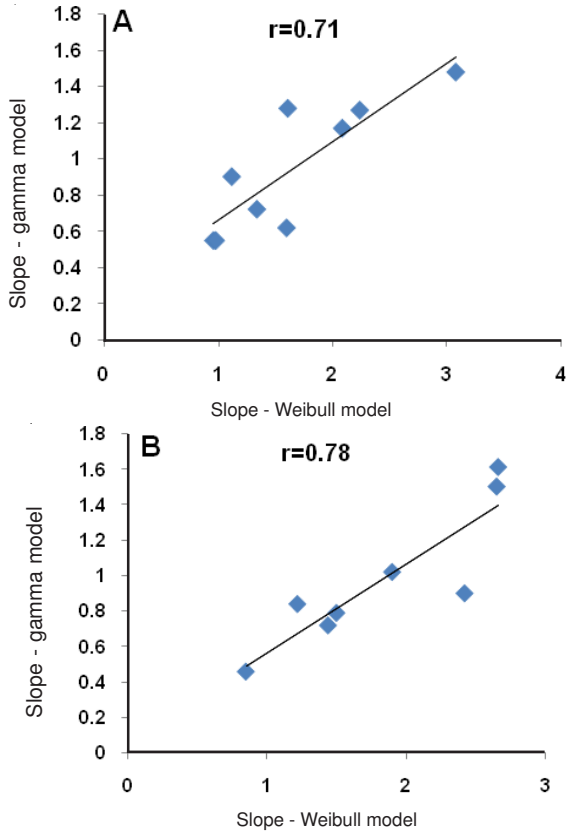

Fig. 9. Comparison of dose/response slopes obtained from tests of 9 chemicals after 30 min (diagram A) and 5 min (diagram B) exposure times at 25 ºC. The slopes were calculated from linear regression equations computed from plots of gamma and weibull mathematical models

Conclusion

The obtained information from calibration test of device and comparison the toxicity results obtained from two models, prove that toxicity test based on bioluminescence will produce similar and repeatable results using other standard distribution models. This research has produced a large amount data on optimization of the best condition for maximum bioluminescence to *Vibrio fischeri*, setting up, calibration of homemade luminometer and evaluation of toxicity of some toxicant and nano materials to *Vibrio fischeri* which had not been investigated up to now.We deduce that there are some differences in the results acquired from two models and results of other investigations.Deviations are chiefly because of differences between laboratory protocols and the method which chemicals are made ready. procedure is reproducible and relatively low cost.

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