www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 8/3 (2015) 304-312

Research article/Araştırma makalesi

The evaluation of the acute toxic effects of Polyvinylferrocenium supported platinum nanoparticles on *Artemia salina* (Brine shrimp)

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Abstract

Elements of platinum group (platinum, palladium) are rarely found in nature, and they spread around the environment only through anthropogenic activities. The environment fails to eliminate them despite the fact that they are used quite a great deal. We studied the effects of Polyvinylferrocenium supported platinum (Pt/PVF^+) nanoparticles, polymer Polyvinylferrocenium (PVF^+) and solid K_2PtCl_4 as comparative depending on concentration and exposure time and mortality on brine shrimp *Artemia salina*. We found that the acute toxicity of these three substances depends on the exposure time and concentration. Toxic effect of all three substances decreased the extension of the duration of exposure. Also, Pt/PVF^+ nanoparticles were toxic Pt/PVF^+ nanoparticles among these three substances at 48 and 96 hour exposure. It was observed that the difference between the PVF^+, Pt/PVF^+ and K_2PtCl_4 concentrations and time groups according to ANOVA multiple comparison test

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Key words: nanotoxicology, *Artemia salina*, acute toxicity, nanoparticles, Polvinylferrocenium

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Artemia salina da polivinilferrosen destekli platin nanopartikülünün akut toksik etkilerinin değerlendirilmesi

Özet

Platin grubu elementler (platin, paladyum) doğada nadir olarak bulunur ve ve sadece antropojenik faaliyetler yoluyla çevreye yayılır. Bu grup elementler oldukça fazla kullanılmasına rağmen çevre bunları yok etmede başarısızdır. Bu çalışmada, indikatör organizma olan tuzlu su karidesi *Artemia salina* da polivinilferrosen destekli platin (Pt/PVF^+) nanopartikülleri, polivinilferrosen (PVF^+) ve katı K_2PtCl_4'ün maruz kalma süresi ve konsantrasyonlarına bağlı olarak ölüm oranı karşılaştırmalı olarak çalışıldı. Bu üç maddenin akut toksisitesinin maruz kalma süresi ve konsantrasyonuna bağlı olduğu bulundu. Bu üç maddenin toksik etkisi maruz kala süresi uzadıkça azalmıştır. Ayrıca, Pt/PVF^+ nanopartikülü bu üç madde içerisinde 48 ve 96 saat maruz kalmada en toksik olarak kaydedildi. PVF^+, Pt/PVF^+ and K_2PtCl_4'ün konsantrasyonları ve zaman gruplar arasındaki fark ANOVA çoklu karşılaştırma testine göre gözlendi.

Anahtar kelimeler: nanoteknoloji, *Artemia salina*, akut toksisite, nanopartikül, polivinilferrosen

1. Introduction

Nanoparticles are the objects with at least one spatial dimension which fall into a nanoscale between 1 and 100 nm. They are grouped, depending on their chemical compositions, as carbon materials, metal oxide, metal nanomaterials, semi-conductors and organic polymeric nanomaterials (Capek, 2010). Nanoparticles have characteristics, such as unique physicochemical properties, high penetration capacity, large surface area and chemical activity. Thanks to these characteristics, they have attracted the attention of industrial and medical technologies. Additionally, nanoparticles are potentially harmful for the environment and the living organisms (Oberdorster *et al.*, 2005; Borm *et al.*, 2006; Kreyling *et al.*, 2006; Lam *et al.*, 2006; Maynard 2007; Krysanov *et al.*, 2010). One of such

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 BioDiCon. 486-0915

nanoparticles is the platinum nanoparticle. The elements of platinum group (platinum, palladium) are rarely found in nature, and they spread around the environment only through anthropogenic activities (of human origin). Today these Pd and Pt nanoparticles are the catalytical elements of automobile catalysts (Johnson Matthey 2009) and are widely used in several technologies and in the field of medicine, as well. The environment fails to eliminate them despite the fact that they are used quite a great deal. Due to the difficulty in eliminating these nanoparticles, there has been a prominent increase in the accumulation levels of the elements of platinum group around the environment (Ek *et al.*, 2004). The contamination by means of the elements of platinum group is caused by the dust on the roadsides, soil, mud and water, notably the particles and substances in the air. This contamination eventually results in the biological accumulation in living organisms (Ravindra *et al.*, 2004 Whiteley and Murray 2003; Zereini *et al.*, 2007: Lepold *et al.*, 2008). It is a known fact that most of the industrial and urban wastes and waste water discharges flow into rivers, lakes and coastal waters. For this reason, it is inevitable for the nanoscaled industrial products and waste products to mix with aquatic environments (Daughton 2004). Moreover, besides the fact that there is little information as to the insufficiency of the environmental monitoring techniques, the difficulties in distinguishing the normal and nano forms of materials, and the emission and the amount of nanoparticles around the environment, the environmental concentrations of nanoparticles vary between μg/L with today's modeling techniques (Boxall *et al.*, 2007). The fact that nanoparticles flow into aquatic environments lead to new environmental problems that need investigating. Therefore, the subjects to be investigated in the first place should be as such the hydrodynamic behaviors of small particles, the relationship between the nanoparticles and larger sediments and colloid particles, lipophilic organic compounds and their binding properties to metals, the synergistic effect that occurs through chemical contaminants and causes toxicity to increase, the ways of nanoparticle intake by living organisms, particle magnitude and their surface effects (Moore 2006).

The studies conducted so far have suggested that suspended solid contents within different aquatic environments play a major role in the differentiation of chemical contaminants and their transportation to remote distances. Separately, the hydrodynamic and morphological characteristics of rivers and coastal regions also determine the distribution boundaries of nanoparticles (Smedes, 1994). The seawater micro-layer in a marine and coastal environment containing sugar, protein and lipid play an important role in the behaviors of nanoparticles. There is no doubt that this situation affects the behaviors of nanoparticles in the aquatic ecosystem and their capacity to be biologically utilized (Wurl and obbard 2004). The ways of nanoparticle intake by aquatic organisms take place through absorption, gills, olfactory organs and their transmission over the skin through to the body wall. The cellular internalization of eukaryotic organisms like protista and metazoan (<100) and the microscopic particles (100 nm-100.000 nm) occurs via endocytosis and phagocytosis (Pelkmans and Helenius 2002; Reiman *et al.*, 2004). In the invertebrates, the cellular immune system along as well as the intestinal epithelium and hepatopancreas are the possible target areas for the swallowed nanoparticles (moore 1990). In the aquatic toxicity studies, the selection of the test organisms is of great importance. In order to obtain updated and significant results from the toxicological tests, it is required that not only the type of test but the appropriate test organisms be selected, as well (Rand, 1995). Previously, while types of fish were simply being selected, now the invertebrates proved to be more sensitive are being picked. In general, the characteristics sought in such organisms are, in short, as follows: The organism to be selected to that end must have an ecological and economic importance, and it must display a broad geographical distribution and must be found any time of the year when required. Also the selected organism must be sensitive to chemical substances, and the individuals used in this respect must be unaffected by parasites and diseases in general as well as being free from any harm; in addition, adequate amount of biological and physiological information must be gained as to the species to be used, and finally, the experiment to be applied has to be appropriate for that species (Bat and Raffaelli 1998). Again, such organisms must have a wide sensitivity as much as possible when they exhibit a sensitivity difference within and among the species. (Greenberg et al., 1985; Rand 1995).

Artemia salina which their typical inhabitants of utter most salty biotopes. The *Artemia salina* (Brine shrimp) is an invertebrate complementary of the fauna the in aquatic ecosystem. *Artemi salina* is significant role in the food chain at marine ecosystem (Lewan *et al.*, 1992). In order to identify the medium lethal concentration (LC_{50}) may be used *Artemia salina* in a laboratory bioassay (Meyer *et al.*, 1982). The necessity for substituting the use of laboratory animals troubling caused by these tests has been increased in the recent years. The different methods include proceedings that could replace the experiments implement with animals used in laboratory test. *Artemia salina* that assay is one of the best and rapid biological and toxicological assays for all laboratory. This bioassay has been exerted to detect general toxicity, in teratology screens, ecotoxicology (Carballo *et al.*, 2002), screening of drugs (Kanwar, 2007) hepatotoxic cyanobacterial strains (Lee *et al.*, 1999), food additives (Kerster and and Schaeffer 1983), and determination of bioactivity of plant product (Carballo *et al.*, 2002; Lopez *et al.*, 2007). It has gained popularity as a test organism because of its ease of culture, short generation time, cosmopolitan distribution and the commercial availability of its dormant cysys (Barahona and Sanchez-Fortun 1999).

Nanoparticles have much more distinct physical and chemical characteristics than the materials known today. Since they have the tendency to cluster within an aquatic setting and to precipitate down to the base, a mobile system should be used in investigating the toxic effects in the aquatic phase. For such reasons, a mobile system should be used and performed for toxic tests. Again, for these reasons, there should be a test system to provide an active setting for toxic tests but to prevent the test organisms from getting influenced by it.

In this study, an aquatic invertebrate organism referred to as *Artemia saliana* (nauplius larva) was used as the test organism. *Artemia salina* was selected due to its special characteristics, such as having a high capacity of adapting to laboratory conditions, being the species easy to keep alive, having sensitivity to chemicals along with their general physiology and well-known behaviors, a short span of life cycle, eggs (cysts) that are easy to supply at any season of the year , and having eggs easy to open in the laboratory; and also, the fact that this species has always been used as an indicator in the toxicity studies makes it the preferable test subject in such researches. In the study we conducted, one of the elements of the platinum group, platinum (Pt) was aided with poly(vinylferrocene) (PVF^+/polymer). Poly (vinylferrocene), which is a redox polymer, is commonly used due to its characteristics, such as having a simple electrochemistry as a basic conductive polymer system, high stabilization and having advantages like being practical in the preparation of thin films by using various methods (Yu *et al.,* 2005; Çelebi, 2008). The toxicity of the basic compound, K_2PtCl_4, from which PVF^+, PVF^+ aided Pt nanoparticle (Pt/PVF^+) and Pt were obtained in the same concentrations, and the assessment of the LC_{50}value in *Artemia salina* along with the mortality rates, depending on the time and concentration, were comparatively evaluated.

2. Materials and methods

2.1 Test chemicals

Poly(vinylferrocenium)-supported pt nanoparticles were prepared according to the procedure described in the literature (Çelebi 2008). K_2PtCl_4 ($\geq$98.2%, Merck) was used as received.

2.2 Preparing of test organisms

Artemia salina cysts (salt lake aqua feed premium *artemia* cysts) were incubated in artificial seawater. The seawater was acquired by using artificial ocean salt. After the artificial sea water was put into use, it was left to rest for a day in the laboratory and was filtered with a 30-µm milipore cellulosic filter. *Artemia* in cyst form were hydrated in distilled water at 4 C° for 12 hours, and was washed to separate the sunken cysts from the floating cysts. The sunken cysts were washed in deionized waster and collected by a funnel or pipette. 3 grams of precleaned cysts were incubated in conic plastic bottles containing 1.5 liter seawater at 30±1C° and *Artemia* hatched in 24 hours. Continuous sunlight was provided with fluorescent lamps. *Artemia* count was done according to the procedure determined by Sorgeloos (1980). For every study to be done, the *Artemia* count was done separately. In short, 100 ml solution containing hatched *Artemia nauplii* larvae was placed in a clean beaker. This solution was constantly mixed to maintain homogeneity, and 1ml of the stock solution was diluted to 100 ml with seawater. This new solution was also continuously mixed; 0.1ml of this solution was taken and placed on a petri dish for counting. The *nauplii* count was determined with this volume under a stereo microscope (Leica S8APO). This count was done separately for each elimination, accumulation, and LC_{50} tests.

2.3 Preparing the Aqueous Suspension of Pt/PVF^+, PVF^+ and K_2PtCl_4

In order to prepare the stock solutions in desired concentrations, the test materials Pt/PVF^+, PVF^+ and K_2PtCl_4composites powders were dispersed in deionized water. Then this solution was vortexed for 30 seconds, and ultrasound water bath was used (Bandelin, sonorex) to increase dispersion and to provide maximum distribution of nanoparticles. After these steps, the determined concentrations were obtained by diluting the stock solution.

2.4 Experimental Setup

Nanoparticles have different physical and chemical properties than those of conventional materials. As they are prone to aggregate and sink in the aqueous medium they are in, motion systems must be used to study their toxic behavior. For this reason, a new test system that provides motion but does not affect the test subjects needs to be developed. For this purpose, 2 liter conic polyethylene bottles were used in exposure tests. A hole was punctured so that thin plastic tubes could enter. To prevent liquid discharge from the lid, it was sealed with silicone and parafilm. To control the air supply and to ensure easy collection of the *artemia*, a valve was placed. Aeration was provided by an aquarium air tank for five bottles were aerated 100 ml for the LC_{50} tests. In this way, a constant mixing was provided without harming the organisms and constant oxygen was provided for *artemia*. In order to use the air pump in more than one bottle, a thin tube was put in the motor's exit, the free end of this tube was connected by a T, and a check valve was placed in order to prevent return flow. At the same time, a plastic lid was placed on top of the system to prevent water evaporation. These systems were used in the 24 and 96 hour test period. The water wasn't replaced during the experiment and no intervention was done.

2.5 Determining the Acute Toxicity of Pt/PVF^+, PVF^+ and K_2PtCl_4

Acute exposure test for *Artemia nauplii* was carried out according to the OECD 202 test guide. The determined concentration of Pt/PVF^+, PVF^+ and K_2PtCl_4 were applied on the *Artemia* culture. The control groups were formed

without the testing compounds. The experiments were carried out in conic plastic containers (0.5 volume) with three repeats. The exposure tests for LC_{50} experiments were carried out in 100 ml volume. For LC_{50}, 50 individuals that were most mobile under the microscope and with the healthiest extremities were chosen. In all concentrations being studied, the dying beings (*nauplii*) were counted under the stereomicroscope at 24, 48, 72 and 96th hours. The experiments were carried out in three repeats. To prevent the nanoparticles sedimentation, aeration throughout the exposure was provided by aeration tube connected to the bottom of the conic bottle. The experiments were carried out in a 16 hour light, 8 hour dark medium at 24±2C°. The pH of the media was measured before and after the experiments, and the mean value was calculated as 7.80 ±2. The *Artemia* were not fed during the exposure tests. After the acute toxicity tests, the changes that took place in the larvae (*nauplii*) exposed to nanoparticles were recorded with the help of analyses under the phase contrast microscope (Nikon Eclipse 80 i). Every exposed group was compared to the control group and the potential anomalies were recorded by taking pictures.

3. Results

The LC_{50} values of Pt/PVF$^+$ nanoparticle, PVF$^+$ and K_2PtCl_4 that were calculated through the 48 and 96-hour-probit analysis and their regression graphics are shown below. Throughout the experiment/test, no mortality or behavioral abnormalities were observed in the control group.

Table 1: LC_{50} values for 96 and 48 hour calculated to Pt/PVF$^+$

Points	48 hours values	96 hours values
LC/EC 1.00	0.000	0.000
LC/EC 5.00	0.000	0.000
LC/EC 10.00	0.000	0.000
LC/EC 15.00	0.003	0.000
LC/EC 50.00	**8.490**	**0.117**
LC/EC 85.00	23615.16	1437.287
LC/EC 90.00	154194.65	13345.071
LC/EC 95.00	2485596.25	362430.781
LC/EC 99.00	457025792.00	177270416.000

As shown in Table 1, in the acute toxicity study conducted at the same concentrations for Pt/PVF$^+$, when we evaluate comparatively the lethal concentration values determined for 48th and 96th hours, the $LC_{1,5,10}$values for 48 hoursand $LC_{1,5,10,15}$ values for 96 hours as the exposure period of nano Pt/PVF$^+$ could not be calculated. While LC_{50}value for 48 hours was 8.490 mg/l, this value regressed to 0.117 mg/l in 96 hours. When the exposure time extends from 48 hours up to 96 hours, 620 times decline is seen in the LC_{50} value (the concentration that kills 50% of living organisms),which suggests that along with the prolongation of the exposure period of Pt/PVF$^+$ nanoparticle, its toxic effect increases a great deal.

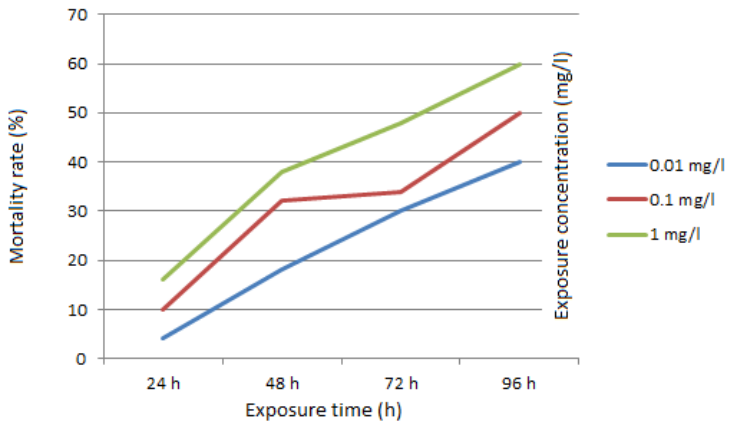

Figure 1: Mortality rates of Pt/PVF$^+$ on *Artemia salina* depending on time and duration

According to the time and exposure period of Pt/PVF$^+$, there is a linear increasing in 0.01 mg/l concentration in the mortality rate graphic of *Artemia salina*, and in the 0.1 mg/l concentration, however, the mortality rate remained unchanged between 48 and 96 hours, and when the exposure time extended up to 72 hours, another increase occurred.

On the other hand, in 1 mg/l concentration, there was a linear increasing that started from 24 hours and extended up to 48 hours; and there was another linear increasing after 48 hours which was, however, less than the increase in 24 hours. The mortality rates are 40% for 0.01 mg/l, 50% for 0.1 mg/l and 60% for 1 mg/l (Figure 1).

Table 2: LC_{50} values for 96 and 48 hour calculated to K_2PtCl_4

Points	48 hours values	96 hours values
LC/EC 1.00	0.001	0.000
LC/EC 5.00	0.030	0.001
LC/EC 10.00	0.161	0.004
LC/EC 15.00	0.505	0.015
LC/EC 50.00	**62.592**	**3.029**
LC/EC 85.00	7760.010	612.598
LC/EC 90.00	24272.963	2151.312
LC/EC 95.00	131499.375	13835.080
LC/EC 99.00	3127743.750	453918.750

As shown in Table 2, in the acute toxicity study conducted at the same concentrations for $K_2Pt\ Cl_4$, when we evaluate comparatively the lethal concentration values determined for 48th and 96th hours, the $LC_{1,5}$ value of $K_2Pt\ Cl_4$ for 48 and 96- hour-exposure periods could not be calculated. Whereas the LC_{50}value in 48 hours was 62.592 mg/l, this value declined to 3.029 mg/l in 96 hours. In order for the $K_2Pt\ Cl_4$ to be able to show its toxic effect in 48 hours as it was shown in 96 hours, the amount of it must increase 20 times more.

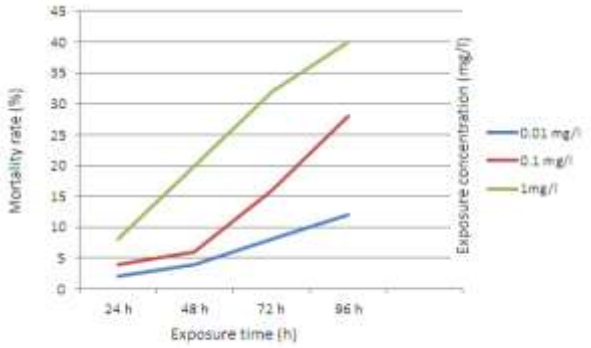

Figure 2: Mortality rates of K_2PtCl_4 on *Artemia salina* depending on time and duration

In the mortality rate graphic of K_2PtCl_4 according to time and exposure periods, there was a little increase in the mortality rate in 0.01 mg/l concentration in the first hours of exposure. After 48 hours, however, there was an ongoing increase until the end of 96 hours. The mortality rate in 0.1 mg/l concentration declined, again, in the first hours as it did in 0.01 mg/l concentration, and an ongoing increase occurred after 48 hours. On the other hand, in 1 mg/l concentration, there was a continual increase in the mortality rate, starting from the first hours of exposure; however, this increase occurred by gradual decrease after 72 hours. Again, the mortality rates at the end of the exposure period were 12% for 0.01 mg/l, 28% for 0.1 mg/l and 40% for 1 mg/l (Figure 2).

Table 3: LC_{50} values for 96 and 48 hour calculated to PVF^+

Points	48 hours values	96 hours values
LC/EC 1.00	0.000	0.000
LC/EC 5.00	0.000	0.000
LC/EC 10.00	0.003	0.001
LC/EC 15.00	0.027	0.002
LC/EC 50.00	**242.67**	**0.150**
LC/EC 85.00	2222129.25	13.858
LC/EC 90.00	19234208.00	40.462
LC/EC 95.00	470791552.00	197.932
LC/EC 99.00	189476962304.00	3887.560

As shown in Table 3, When we comparatively evaluated the lethal concentration values of the practices performed at the same concentrations for PVF^+ which were determined for 48 and 96- hour-exposure periods, the $LC_{1,5}$ values of PVF^+ for 48 and 96 hours could not be calculated. Whereas the LC_{50} value for 48 hours was 242.67 mg/l, it was calculated as 0.150 mg/l for 96th hour, because the amount of it must increase 1.613 times more in order for the toxic effect shown at the 96th hour to emerge.

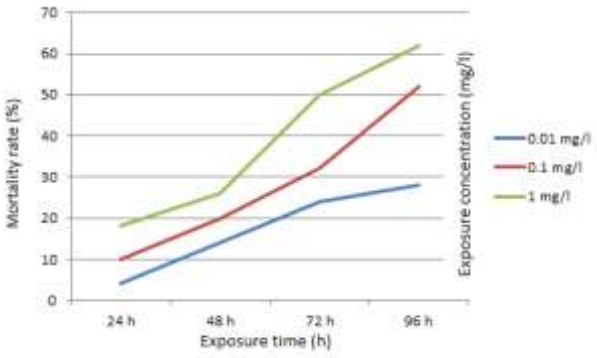

Figure 3: Mortality rates of PVF^+ on *Artemia salina* depending on time and duration

In the mortality rate graphic of PVF^+ according to the time and exposure periods of *Artemia salina*, the mortality rate always showed an increase with the prolongation of exposure period in 0.01 mg/l concentration. However, this increase continued to decline gradually after 72 hours. There is also a continual increase in 0.1 mg/l concentration as in 0.01 mg/l concentration; yet, contrary to 0.01 mg/l, this increase continued more and more after 72 hours. In 1 mg/l, however, the increase continued with the prolongation of concentration period. The mortality rates are 28% for 0.01 mg/l, 51% for 0.1 mg/L and 61% for 1 mg/l (figure 3).

3.1 Analysis of exposure durations and concentration groups

For all concentration groups of PVF^+, the highest difference was created by 1 mg/l and 96 hours of exposure duration (Table 4).

Table 4: The results of Tukey test with respect to differences between mortality rates based on varying concentration and exposure durations to PVF^+

Hours	Subset for alpha = 0.05			Concentration	Subset for alpha = 0.05		
	1	2	3		1	2	3
24-h	0.0783[a]			0* mg/l	0.0000[a]		
48-h	0.1500[ab]	0.1500[ab]		0.01 mg/l		0.1733[b]	
72-h		0.2633[bc]	0.2633[bc]	0.1 mg/l		0.2817[cb]	0.2817[cb]
96-h			0.3567[c]	1 mg/l			0.3933[c]
Sig.	0.728	0.366	0.535	Sig.	1.000		0.188

0* mg/l: control group

Table 5: The Difference between the PVF^+ concentration and time groups according to ANOVA multiple comparison test

(I)Hours (h)	(J)Hours (h)	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
24-h	72-h	0.18500*±0.06897	0.049	0.3691	0.0009
	96-h	0.27833*±0.06897	0.001	0.4625	0.0942
48-h	96 h	0.20667*±0.06897	0.022	0.3908	0.0225

(I) Con. (mg/L)	(J) Con. (mg/L)	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	UpperBound
1	0.01	0.22000*±0.05466	0.001	0.0740	0.3660
	0*	0.39333**±0.05466	0.000	0.2474	0.5393
0*	0.01	0.17333**±0.05466	0.014	0.3193	0.0274
	0.1	0.28167**±0.05466	0.000	0.4276	0.1357
	1 mg/l	0.39333*	0.000	0.5393	0.2474

0* mg/l: control group, con.: concentration, sig.:significance

According to Anova results between mortality rates due to concentrations of PVF^+ at the end of the exposure duration, significant differences at the level of $P<0.05$ were found between concentration intervals 0.01mg/l – 1 mg/l and 0.01mg/l -0* mg/l whereas another significant difference at the level of $P<0.01$ was found between concentrations of 0*mg/l and 1 mg/l. Between mortality rates due to concentrations of PVF^+ at the end of the exposure duration; significant differences at the level of $P<0.05$ were found between exposure durations of 24h-72h, 24h-96h and 48th and 96th hour groups (Table 5). The group which made the most difference in all the concentration and time groups of Pt/PVF^+ was the 1mg/l concentration and 72 hours (Table 6).

Table 6:The tukey test results of the differences in the mortality rates between the different concentration groups and exposure time of Pt/PVF^+

Hours (h)	Subset for alpha = 0.05	Concentration (mg/L)	Subset for alpha = 0.05			
			1	2	3	4
24-h	0.0767[a]	0* mg/l	0.0000[a]			
48-h	0.2217[a]	0.01mg_l		0.4067[b]		
96-h	0.3800[a]	0.1mg/l			0.4933[c]	
72-h	0.5067[a]	1mg/l				6200[d]
Sig.	0.084	Sig.	1.000	1.000	1.000	1.000

0* mg/l: control group

Table 7: The Difference between the Pt/PVF^+ concentration groups according to ANOVA multiple comparison test

(I) con. (mg/L)	(J) con. (mg/L)	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
0.01	0,1	0.08667*±0.01054	0.000	0.1204	0.0529
	1	0.21333*±0.01054		0.2471	0.1796
	0*	0.40667*±0.01054		0.3729	0.4404
0.1	0,01	0.08667*±0.01054	0.000	0.0529	0.1204
	1	0.12667*±0.01054		0.1604	0.0929
	0*	0.49333*±0.01054		0.4596	0.5271
1	0.1	0.12667*±0.01054	0.000	0.0929	0.1604
	0*	0.62000*±0.01054		0.5862	0.6538

0* mg/l: control group, con.: concentration, sig.:significance

According to the ANOVA results, among the mortality rates of Pt/PVF^+ depending on the concentration at the end of the exposure period, significant differences were found between all the concentration groups and the other concentration groups at $P<0.01$ level. There is no difference between the time groups in terms of the mortality rates of Pt/PVF^+ at the end of the exposure period (Table 7). The group which made the most difference in all the concentration and time groups of Pt/PVF^+ was the 1mg/l concentration and 96 hours (Table 8).

Table 8: The tukey test results of the differences in the mortality rates between the different concentration groups and exposure time of K_2PtCl_4

Concentration	Subset for alpha = 0.05			Hours (h)	Subset for alpha = 0.05	
	1	2	3		1	2
0* mg/l	0.0000[a]			24-h	0.0283[a]	
0.01 mg/l	0.0667[ab]	0.0667[ab]		48-h	0.0883[ab]	0.0883[ab]
0.1 mg/l	0.	0.1317[b]		72-h	0.1383[ab]	0.1383[ab]
1 mg/l			0.2550[c]	96-h		0.1983[b]
Sig.	0.226[abc]	0.246[abc]	1.000[abc]	Sig.	0.089	0.089

0* mg/l: control group

Table 9: The Difference between the K_2PtCl_4 concentration groups according to ANOVA multiple comparison test

(I) Con (mg/L)	(J) Con. (mg/L)	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
0.01	1	0.18833*±0.03433	0.000	0.2800	0.0967
0.1	1	0.12333*±0.03433	0.004	0.2150	0.0317
	0*	0.13167*±0.03433	0.002	0.0400	0.2233
(I) Hours	(J) Hours	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
24-h	96-h	0.17000*±0.04555	0.003	0.2916	0.0484

0* mg/l: control group, con.: concentration, sig.:significance

According to the ANOVA results, among the mortality rates of K_2PtCl_4 depending on the concentration at the end of the exposure period, there were significant differences determined between 0.01 mg/l concentration and 1 mg/l concentrations at $P<0.05$ level, and between 0.1 mg/l concentration and 1 mg/l, and between 0.1 mg/l and 0* mg/l concentrations at $P<0.05$ level. Among the mortality rates of K_2PtCl_4 depending on the hour groups at the end of the exposure period, significant differences were found between 24 hours and 96 hours at $P<0.05$ level (Table 9).

4. Conclusions and discussion

Table 10: The LC_{50} values obtained from the tests performed at the same concentrations

	LC_{50} values	
	48 hours	96 hours
Pt/PVF^+	8.490	0.117
PVF^+	242.67	0.150
$K_2Pt\ Cl_4$	62.592	3.029

When we examine the table 10, in the studies carried out at the same concentrations, the lethal concentration values of Pt/PVF^+ nanoparticle, PVF^+ and K_2PtCl_4 assessed for 48 and 96 hours were compared, the $LC_{1,5,10}$ values of the nano Pt for 48 and 96 hours and LC_{15} values for 96 hours could not be assessed/calculated. Considering the toxic effect demonstrated by each of these three substances within the 48-hour-exposure period, it can be observed that the most toxic one is Pt, whereas the least toxic one is PVF. Considering the toxic effect shown at the 96th hour, it is seen that the most toxic substance was Pt, while the least toxic one was K_2PtCl_4. We can also say that during the 96-hour-exposure period, again, the most toxic one was Pt/PVF^+ nanoparticle, which was followed by PVF^+, and then the least toxic one was K_2PtCl_4 by taking into consideration the LC_{50} values. On the other hand, when the exposure period extended from 48 hours upto 96 hours, the most toxic effect was demonstrated by PVF^+, since theLC_{50}value had increased by1.613 times, which was followed by Pt with an 28 time- increase and K_2PtCl_4 with 20 time- increase. According to these results, with the prolongation of the exposure period of each of these substances in general, we can state that the toxic effect was on the increase and that this toxic effect of PVF^+ particular, considering the others, had extremely increased as regards the exposure time (Table 10).

Table 11: Rate mortality according to concentration (%)

	Mortality rate (%)		
	0.01 mg/l	0.1 mg/l	1 mg/l
K_2PtCl_4	12	28	40
Pt/PVF^+	40	50	60
PVF^+	28	51	61

When we examine the table 11 above, it is clear that the mortality rate increased along with the increase in concentration for each of the three substances. Considering the 0.01 mg/l concentration, the mortality rate in the Pt/PVF^+ nanoparticle is rather high when compared to the others. In the 0.1 and 1 mg/l concentration, however, the Pt/PVF^+ nanoparticle and PVF^+ mortality rates are the same.

Consequently, the difference in the mortality rate between the concentrations of Pt and PVF^+ nanoparticles is not that much; yet, the mortality rate of PVF^+ and K_2PtCl_4 depends on the concentration. The Pt/PVF^+ nanoparticle is more toxic than the others. Separately, in the graphicon *Artemia salina* showing the mortality rate that depends on time and concentration, K_2PtCl_4 and PVF^+ yielded a monotonous reaction (the expected response), whereas the Pt/PVF^+ nanoparticle, when both the concentration rates and the exposure time were taken into account, showed a rather a distinct character (rough/fluctuating), which also indicates the fact that when the substances are reduced to nano-dimensions, they may exhibit very distinct characteristics.

In conclusion, it was reported that the toxic effect of the nanoparticle reported previously by several researchers had varied by depending on a number of parameters, such as the nanoparticle size, the way of synthesis and the surface where it is synthesized, in addition to which its toxic effect generally increased when substances were reduced from micro dimensions down to nano dimensions. In order to confirm the same previously-reported information in this study, we evaluated the acute toxicity of Poly(vinylferrocenium), Pt nanoparticle aided with Poly(vinylferrocenium) (the surface it was synthesized on) and the compound K_2PtCl_4, from which the Pt nanoparticle was obtained, on *Artemia salina*.

As the result of this evaluation, it was concluded that considering the exposure time and the concentration, the nanoparticle had a more distinct toxic effect than the surface it was synthesized on, and again, that the nanoparticle had a more toxic effect than the compound it was obtained from.

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(Received for publication 07 September 2015; The date of publication 15 December 2015)