SYNTHESIZING, CHITOSAN COATING AND DETECTING THE NANOTOXIC EFFECT OF THE LEAD SELENIDE (PbSe) QUANTUM DOTS

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Researches on nano-structured materials (<100 nm) are increasing rapidly in the direction of technological developments. Synthesis and modification of quantum dots, known as nanomaterials and nanocrystals, have become an important aspect of nanotechnology. The reason for this is that it has many potential applications in the field, including biomedicine and pharmacology. The unique properties and biological effects of nanostructured particles indicate that they can potentially be used as an alternative in the treatment of various diseases. In this work, PbSe quantum dots were synthesized by chemical method and modified by chitosan coating. The characterization of PbSe was done by TEM, SEM, XRD, DLS and Zeta potential analysis methods. When the morphological structures of the quantum dots obtained by chemical synthesis were examined, round and spherical nano-sized particles (<20 nm) of all PbSe were obtained according to TEM and SEM results. It has been seen that PbSe quantum dots have a positive (+) surface charge and are highly stable (40 + mV). In addition, the increase in oxidation resistance of PbSe coated with chitosan was found in XRD results. As a result of this research, small size and biopolymer coated and thus more stable quantum dots could be obtained. It has also been found that PbSe quantum dots coated with biopolymers like chitosan may have the potential to be used in pharmacology and biomedical applications.

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Keywords: Lead Selenide, Quantum Dots, Synthesis, Chitosan Coating, Nanotoxicity

1. Introduction

Due to the very small size of the nanostructured materials, nanoparticles (especially those smaller than 20 nm) display excellent optical, magnetic and chemical properties [1]. Nowadays, nano scaled materials has been the fastest growing area in parallel with the development of new technologies in areas such as biomedical research, electronics and entertainment sector, energy production and storage, environmental health and hygiene sectors. Despite the fascinating properties of nano-structured materials, very little is known about the side effects on human life and the environment. Problems with the designed nanomaterials come to light as nanoparticles or products made of quantum dots enter into human life [2-5]. Many studies on the determination of possible toxicological properties of nanostructures with novel properties, or production of biomaterials of high biocompatibility are currently being investigated as research topics of nanotechnology. Since the problems associated with the potential impact of nanomaterials on human health and the environment have just emerged, our knowledge of the environmental and human health effects of nanotechnology is still very poor [1,5].

Quantum dots are semiconductor crystal materials with sizes ranging from 2 to 10 nm and are particularly attractive nano materials for optical work [6,7]. Their greatest feature is that they are one of the first to be applied to biological sciences in the fields of nanoscience and nanotechnology. Quantum dots are extremely effective in fluorescence displaying of biological tissues and it is an important area where continuous research and development is being made for the detection and diagnosis of diseases, fluorescence measurement-adjustment in development new

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drugs, monitoring single protein and examination of cells. It has been observed that optical and electronic behaviours and properties of extremely small size semiconductors change depending on grain size. For these reasons, quantum dots have become one of the important research topics. Especially in biology and biomedicine fields, there is a potential for significant use [6,8]. Highly fluorescent single-scatter quantum dots are commercially available. It can be sent to biological targets by surface alignment and by pairing with antibodies, peptides or small molecules. There are three basic elements in creating a quantum dot. These are the synthesis of quantum dots; modifying the surfaces, making them biologically compatible, and further processing the surface so that the dots can be directed to the target tissue. Quantum dots are directed to biological target tissues by adjusting them with antibodies, peptides or small molecules [7].

Lead selenide (PbSe) quantum dots absorb infrared light and are suitable for both renewable energy systems and optical devices. Semiconductor PbSe quantum dots have high absorption characteristics in infrared (IR) and near IR range. Because of these properties, they are important materials in photovoltaic applications and solar energy transformation [9-11]. The bulk of these materials are toxic due to heavy metals such as lead. Selenium also causes toxicity at high levels but is nutrient for organisms at low concentrations. At present it is not known whether the PbSe quantum dots are indeed toxic to aquatic organisms. Because of this reason, Pb release is an important concern. They are usually coated with silica [12] and zinc sulphide [13] to prevent dissolution of the nucleus of these quantum dots. They are said to be harmless with such coatings based on cell structure studies [14,15]. The results from the culture studies are informative but are not sufficient to understand the effects on aquatic species and different environments. Exposure of aquatic organisms to these materials can lead to unprecedented and undesirable consequences, since the stability, accumulation limits and toxicity of these species are not fully understood.

The environmental concern associated with nanomaterials or quantum dots is that the aquatic organisms in the food chain are exposed to them. The most important organisms that form the food chain in this biological system are undoubtedly aquatic organisms. The topic in concern is that a significant part of people's food is the aquatic organisms. These organisms (zooplankton etc) eat living creatures (phytoplankton, etc.) contaminated with nano-structured particles alongside the transported organic and inorganic particles. Nano toxic effects are difficult to fully appreciate because the effects observed depend on the size, physico-chemical properties, environment and organism diversity of the nanoparticles or quantum dots. Since the toxicity tolerances of organisms vary widely, the possible toxic properties of nano-structured materials explain most of the differences. In this work, thio-stabilized PbSe quantum dots in aqueous solution were synthesized and their Nano toxic effects were investigated. In addition, chitosan-coated PbSe were obtained and characterized.

2. Experimental section

2.1. Chemical materials

Chemicals such as lead, selenium, thioglycolic acid, dimethyl sulphoxide, ethanol, sodium hydroxide chemicals and chitosan (poly-[β-(1,4)-2-amino-2-deoxy-β-D-glucopyranose]) chemicals which we use in our research are procured from Sigma Aldrich. All chemicals from the analytical reagent class were used without further decontamination or purification.

2.2. Synthesis of lead selenide quantum markers

It was obtained by using lead acetate trihydrate $Pb(Ac)_2 \bullet 3H_2O$ (99.0-103%) and selenium dust (99% +) aqueous solution according to the procedure described by Primera-Pedrozo et al. [16] for the synthesis of thiol-stabilized PbSe quantum dots. For synthesis, 0.190 g of lead acetate dissolved in 250 mL of deionized water in a three-neck flask. Then, 1.0 mL of TGA, MPA solutions were added under stirring. The pH of the solution was adjusted to pH 10.0 for TGA with 1.0 M NaOH. Selenium (IV) solution (2.5 mL of 0.5 M) was placed in a second three-neck flask, the flasks were connected through a teflon tubing (4 mm i.d.) and the solutions were deaerated by purging N_2 for 30 min. Then, 10 mL of 10% $NaBH_4$ stabilized in 0.1% NaOH solution was added

to the Se (IV) solution. The H_2Se gas produced was swept into the Pb-thiolate solution under N2 flow yielding dark PbSe nanocrystals rapidly. Nitrogen was purged for an additional 20 min. Stirring was kept at room temperature for an hour to ensure stable formation of stable nanocrystals.

2.3. Use of chitosan biopolymer for coating

Chitosan, a natural biopolymer, has maintained its place as an interesting material for researchers in the last 50 years. Chitosan, which has many advantages compared to chitin, has found use in many branches of industry, especially in food, cosmetics, agriculture, medicine, paper and textile. The chemical structure of chitosan is poly[β-(1,4)-2-amino-2-deoxy-β-D-glucopyranose]. Chitin and chitosan polysaccharides chemically resemble celluloses but show some differences among themselves. There is a group of hydroxyls (-OH) bound to the second carbon atom in cellulose, while there is acetamide ($-NHCOCH_3$) in the chitin and amine ($-NH_2$) group is in the chitosan. Chitosan has a total of three reactive groups, the primary (C-6) and secondary (C-3) hydroxyl groups in each repeating unit and amine (C-2) group. These reactive groups can easily undergo chemical modification and change the mechanical and physical properties and solubility of the chitosan. The coating process using chitosan is summarized as follows; One gram of 75-85% deacetylated chitosan with medium mole mass (200-500 kDa) was dissolved in 100 mL of 0.5% (v / v) acetic acid containing solution. 80 mg of PbSe nanoparticle suspended in 50 mL of ethyl alcohol was added to this solution. This mixture was stirred with a magnetic stirrer for 24 hours at high intensity and centrifuged at 4100 rpm for 90 minutes. The supernatant was removed, the pellet was washed twice with ethyl alcohol and the product was dried.

2.4. Bioassay organism

Artemia salina organism used in the experiment is a kind of arthropod zooplankton living in salt water and salt lakes. Also known as saltwater shrimp, it is an organism resistant to a wide salinity range (1-200 ppt). Artemias can grow up to 2 cm in their natural environment. They are usually fed by filtering microscopic size phytoplankton in the water. They are known as a valuable source of live nutrients in the food chain, especially in the feeding of seed fish. They are one of the easiest zooplankton organisms, which have high nutritional value in protein, to produce in the laboratory environment. Artemias reproduced in culture environment namely almost all the offspring (nauplii) after the incubation process are very important for toxicological or biotechnological studies since they do not contain any parasites, and most importantly, they are of the same size and specificity [17]. The incubation process of artemia eggs, commercially available and capable of being stored for a long time without deterioration, is briefly as follows; Put about 2-3 gr Artemia eggs in 1 liter of sea water (‰ 30 ppt). Since Artemia eggs are grown in a basic medium, some carbonate should be added into the water if the pH level is below 7.6. At a temperature of 26-28 ºC, the water must be in continuous circulation with strong air reinforcement by means of an air motor. Under continuous lighting, 95% of the larvae come out within 24-36 hours.

2.5. Characterization of lead selenide quantum dots

Quantum dots were identified using the methods used to characterize materials at the nanoscale. In this direction, characterization analyses of PbSe were performed by using Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), Zeta Potential, Scanning Electron Microscopy (SEM) and X-Ray Diffraction Spectroscopy (XRD) measurement methods.

2.6. Toxicity bioassay method

The toxicity immobilization tests of this study are carried out according to the guidelines of the Organization for Economic Co-operation and Development [18]. Concentrations are known to be important in toxicity tests. In this study, the concentrations of low PbSe quantum dots are

adjusted, especially in the low range. Experimental concentrations to which organisms have been exposed have been made as wide range as possible (between 0.2 and 25 mg / L) (Table 1).

Table 1. Bioassay design of Artemia salina organisms exposed to PbSe.

Groups	Control	Group 1	Group 2	Group 3	Group 4	Group 5
PbSe *	0	0.2	1	5	10	25
A. salina ** (number)	15.000	15.000	15.000	15.000	15.000	15.000
Recurrence	3	3	3	3	3	3

** The concentration of semiconducting quantum dots indicates the ppm ratio (mg / L).*
*** It was initiated by placing 30-35 A. salina organisms in an average of 1 mL in each group in a plastic container with a total internal volume of 500 mL at the beginning of the experiment.*

Artemia culture was exposed to PbSe suspensions at different concentrations. As shown for the acute exposure, it was carried out for short periods of time at a regulated concentration of 0.2-25 mg L-1 (Table 1). A control group was also created for the organism without the test compound. The toxicity immobilization experiments were carried out in 18 conical plastic containers (0.75 L internal volume). Each 500 mL volume level of the container was marked and after filled with seawater separately, the prepared nanoparticle suspensions were added as described above [17]. Ventilation is provided by a line extending to the bottom of the conical containers (Figure 1). During the test, 16:18 hours: a dark light regime and a temperature of 24 ± 2ºC were provided. In order to determine the nanotoxicity rates of the organisms exposed to the PbSe quantum dots, the number of individuals alive in each group was regularly recorded according to exposure times and the obtained data were given graphically.

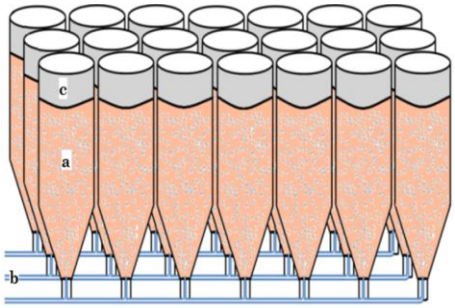


Fig. 1. Artemia salina bioassay system.
a.) The medium in which the test organisms are found with the quantum dots of PbSe
b.) Ventilation system of the test system c.) 500 mL plastic test cones

2.6.1. Counting Artemia salina organisms

The rate of extinction of Artemias vary; so, it is important to count as accurately as possible the number of artemia offspring that have been incubated before the experiment. As in all zooplankton, the amount of population was determined by dilution method in artemias. Briefly, the counting strategy is as the following. 100 mL solution containing incubated artemia was placed in a clean glass beaker. 1.0 mL of this stock has been transferred into 100 mL through continuous mixing and diluted with water to 100 mL. (100-fold dilution). Then, 0.1 mL of this diluted solution was removed while stirring and counted under light [19]. The number of artemias was visually determined in this volume (0.1 mL). Once the required number of artemia for the experiment was set, the organisms were exposed to PbSe at the concentration ratios indicated in Table 1. above.

2.6.2. Phase Contrast Microscopic Analysis

Live artemia samples were collected from all bioenergy groups, including the control group, and the accumulation of the quantum dots of the organisms was visually examined under a phase contrast microscope equipped with a digital camera (Micromaster, Model 12-575-252,

Fisher Scientific). Images from live organisms were recorded by Mikron imaging software. All images were taken on an 80 μm scale to obtain a clear picture of artemias.

2.6.3. Determination of survival rates of experimental organisms

Artemia salina organisms were exposed to PbSe at different rates in order to determine the possible toxicity effects of the PbSe quantum dots obtained in the study. It is important to determine the possible effects of the PbSe quantum dots at different periods, which the organisms are exposed to during the entire period of the bioassay process. Nanoscale materials in the medium may sometimes not show any real effect due to the physical and chemical properties of the medium. Salt water which is toxic in fresh water may not show the same toxic effect or vice versa. The same nanomaterial may not show the same effect at different times of the experiment. Because of all these reasons, it is important to count how the organism affects or how long it lives in different periods (times). In this context, in order to evaluate the living rate of organisms; 1.0 mL of the medium was transferred to a 10 mL test tube and diluted to 10 mL with sea water (10-fold dilution) at the start (0 h), 24th, 48th, and 72nd hours. This diluted solution was then manually mixed, and 0.1 mL was taken and the number of organisms living in the light was visually determined in this volume. For each recurrence the count was made to be three parallel and the average of the numbers was taken.

3. Result and discussion

3.1. Transmission electron microscope analysis results

To obtain TEM images, the diameters of about 100 particles, which have been measured in the 3 microscope images taken from the random areas in the surface in the NP areas on the copper grids coated with carbon, were examined and whether they form aggregates or not is determined. TEM images of the PbSe quantum dots coated with chitosan and the PbSe quantum dots synthesized in the laboratory environment are given in Figure 5-6. Although nanoscale materials exhibit a distinct characteristic, they are known to tend to aggregate in colloidal (aquatic environment) structure. Similar results were obtained in this study. When the shape of the quantum dots of the PbSe is examined, it is seen that the majority of the quantum dots have a round or spherical structure. According to the TEM results of the samples taken immediately after the synthesis end, the quantum dots show a homogeneous distribution between 2-15 nm in size, but usually the amount of aggregation is very small. It is seen that PbSe quantum dots are successfully encapsulated with chitosan. However, it is observed that as the size of the particles increases due to the coating of the chitosan, aggregation is present. It is clear that not all chitosan-coated PbSe quantum dots yield the same result, but in particular particles below 100 nm are obtained.

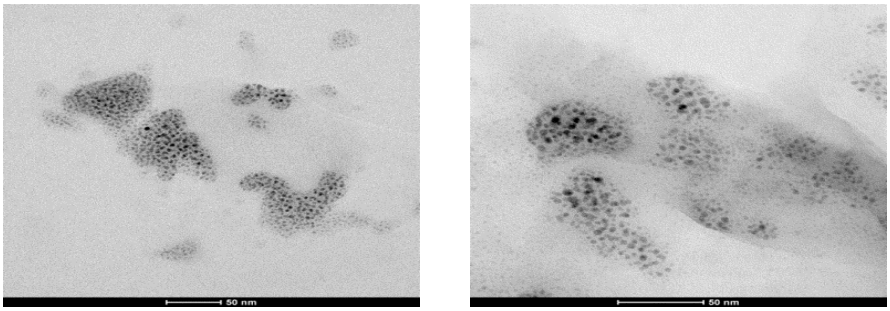


Fig. 2. TEM images of lead selenide quantum dots synthesized in the laboratory environment.

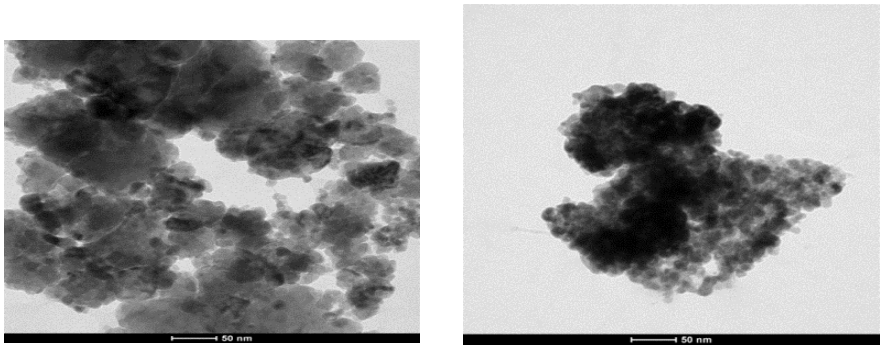


Fig. 3. TEM images of lead selenide quantum dots encapsulated with chitosan

3.2. Dynamic light scatter analysis results

The Dynamic Light Scatterer (DLS) is often used to dimension nanosized particles in a colloidal solution and to determine aggregates in suspensions. The hydrometric diameter (a hypothetical impermeable diameter of a sphere spreading at the same rate with the characterized particles) can be measured in terms of the time dependence of the scattering density measurements. The diameter value obtained is the diameter of the sphere with the same diffusion coefficient as the particle. This diffusion coefficient depends not only on the particle size but also on the type and concentration of ions in the medium. The hydrodynamic diameter is complementary to other size measurements, such as TEM, because it provides information on the aggregation states of the NP solutions. The highly aggregated solution has hydrodynamic diameters much larger than the TEM size, while the nonaggregate colloidal solutions have hydrodynamic diameters similar to or slightly larger than the TEM size. The DLS results (nm) of the PbSe we have obtained in our study are given in Figure 4. When the particle size distribution results are examined, it is seen that the actual sizes of all PbSe grow at a few or 10-100 times. This is a general characteristic of nano-sized particles and it is observed that they are growing in liquid medium. It is also known that quantum dots are very difficult to stabilize in liquid medium. Therefore, as long as the PbSe are in the liquid medium, they form aggregates and the DLS results can be obtained in μm levels depending on the concentration (mg / L).

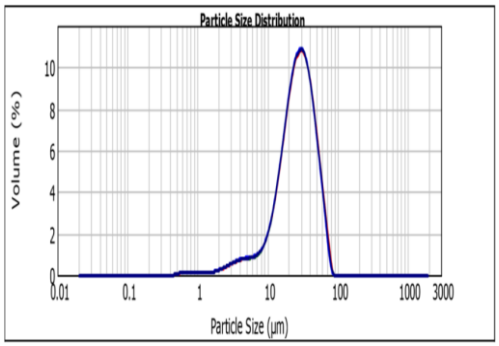


Fig. 4. Particle size distributions of lead selenide quantum dots.

3.3. Zeta potential analysis results

Zeta potential is an important parameter for evaluating the stability of PbSe in aqueous suspensions. Particles having a large negative or positive zeta potential are likely to repel each other, thus increasing the suspension stability. The results of the Zeta potential results show that PbSe's have positive (+) surface load. Particles with a positive zeta potential are bound to negatively charged surfaces or vice versa. The size of the Zeta Potential provides information about particle stability, higher magnitudes of potential display increased electrostatic repulsion and

hence increased stability. For example, 0-5 mV particles display aggregation or tendency to aggregate, 5-20 mV particles are minimally stable, 20-40 mV particles are moderately stable, and particles larger than 40+ mV are highly stable. Accordingly, it can be seen that the quantum dots are highly stable since the PbSe obtained in the study are 40+ mV.

3.1.4. X-ray diffraction analysis results

X-ray diffraction results are given in Figs. 6.a-b. Accordingly, when the X-ray crystallographic patterns of both samples are examined, the characteristic peaks appear to be more pronounced in the chitosan-coated sample. The reason for this is the high oxygen sensitivity of naked PbSe quantum dots. The quantum dots aggregated quickly and synthesized with oxygen and oxide compounds and the impurities found in small quantities are thought to be the cause of this noise. Particularly in the uncoated sample graph, the scattering peaks at 2-theta 26.71◦, 27.97◦, 32.44◦ and 39.82◦ were interpreted as impurities due to the formation of $PbSeO_3$. Immediately after the synthesis, unwanted interference was eliminated in the coating process. The scattered peaks observed at 2-theta, 29.16◦, 43.60◦, 49.31◦, 51.71◦, 62.40◦, 69.55◦, 71.45◦ and 78.05◦ corresponds to the (1 1 1), (2 0 0), (3 1 1), (2 2 2),(4 0 0), (3 3 1), (4 2 0) and (4 2 2) surface respectively. From these data it is understood that the crystal structure of PbSe quantum dots is face-centred cubic. These results were found to be consistent with previous studies on PbSe [20,21].

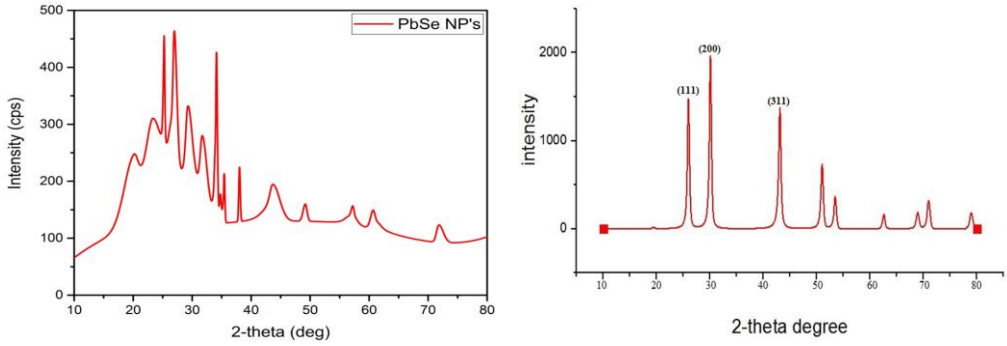


Fig. 5. X-ray diffraction results of lead selenide quantum dots a) PbSe quantum dots b) Chitosan-coated PbSe quantum dots.

3.1.5. Scanning electron microscope analysis results

The SEM results of the PbSe quantum dots are shown in Fig. 7 and the chitosan-coated PbSe SEM results are shown in Figure 8. When the images obtained are examined; PbSe and chitosan-coated PbSe aggregates and some amorphous particles and spherical forms and almost homogenous aggregates are observed. When the SEM results of the uncoated PbSe sample are examined, it is observed that the nanocrystals are moderately homogeneous and spherical, while aggregation is moderate. Both samples formed aggregates, but it was observed that there was more aggregation in the chitosan-coated PbSe quantum dots. Due to this agglomeration, it is difficult to determine the particle boundaries of the coated individual particles. The boundaries of the individual particles coated at a magnification of 140,000 could be determined and found to be in the size range of 40-80 nm. These values are much larger than the value determined by TEM. The reason can be explained by the high rate of agglomeration in aqueous media of both samples. It has been found that the sizes of the quantum dots of both examples are close to each other, and it has been understood that the coating made with the chitosan does not have a detrimental effect on the particle size. Agglomeration form of Chitosan-coated particles have more dense morphology on the surface. In addition, the sizes of the agglomeration aggregates were found to be 1-1,12 μm on average by both SEM and DLS analyses. It has been found that these results are consistent with those previously reported by Achimovicova et al. [22] on PBSI. While the forms of the uncoated sample particles are more homogeneous and polygonal; the PbSe quantum dots coated with chitosan are more homogeneous and spherical.

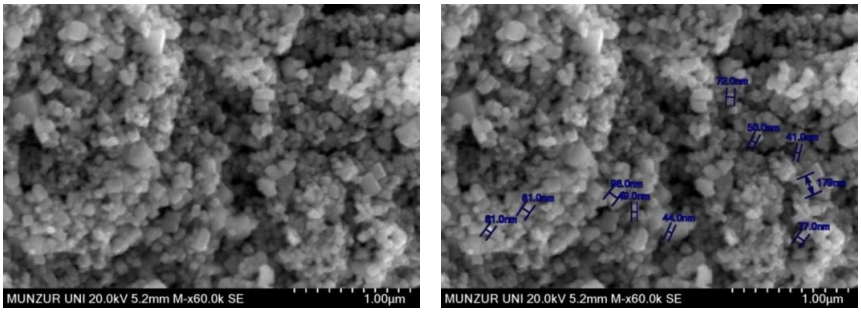


Fig. 6. SEM images of PbSe quantum dots spots.

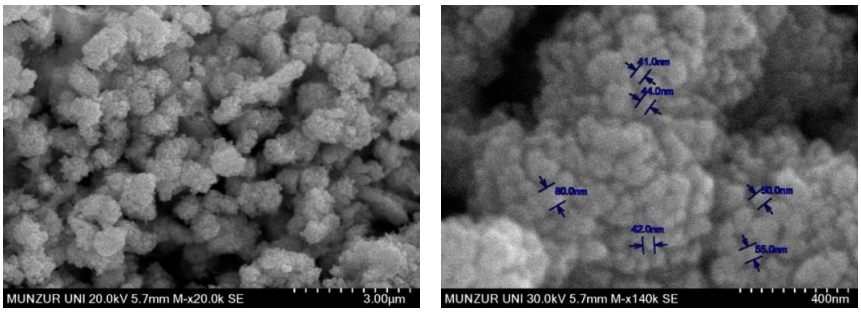
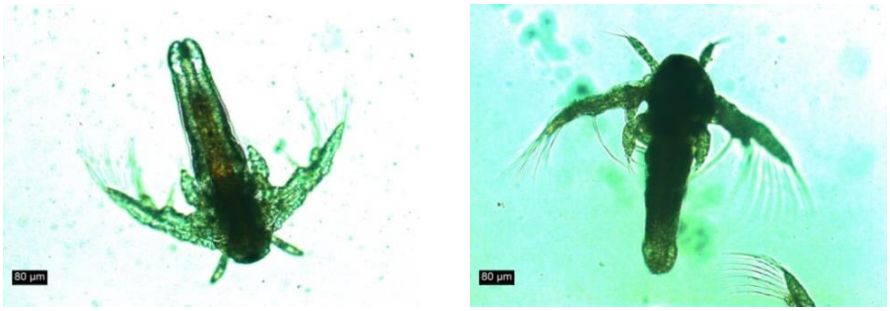


Fig. 7. SEM images of chitosan-coated PbSe quantum dots.

3.1.6. Results of phase contrast microscopic analysis

Phase contrast images of artemias exposed to PbSe quantum dots (25 µg / mL, 72 h) with the control group not subjected to any application are given in Fig. 9. When the phase contrast microscopic results are examined; *A. salina* organisms seem to have received the PbSe in the medium. According to TEM and SEM images, quantum dots formed aggregates. The result is that the vast majority of all particles taken up by the organism are aggregated quantum dots and are easily accumulated by the organism. Because artemia is fed by filtration, it takes all the particles in the micro or macro size present in the aqueous medium to the body as food. When it is taken by the organism and accumulated within it, it creates a danger for the upper consumer. These results are in line with previous studies [17].



a) salina (Control) *b) salina exposed to NPs*

Fig. 8. Artemia exposed to NPs (25 µg / mL, 72 h) with the control group (since pictures of all NP exposed experimental organisms are similar only one group of pictures is shown).

3.1.7. Survival Rates of Artemia salina

The survival rates of *Artemia salina* exposed to PbSe quantum dots are shown in Figure 13. When the survival rates of *A. salina* individuals were examined, it was determined that the highest survival rate was 72.55% at the 48th hour of 10 ppm concentration and the lowest survival rate was 42.50% in the beginning of the control group (Figure 10). When the elimination groups were examined, the highest survival rate was found in the 5-ppm concentration with 31.00% and the lowest survival rate was 14.00% in the control group. When the rates of survival in all the groups in which the practice was conducted were examined, it was seen that the highest data were generally at a concentration of 10 ppm. The survival rates obtained showed an increase up to a concentration of 10 ppm in parallel with the increase in concentration and then showed a decreasing tendency. Within each group, it was observed that the survival rates obtained according to the duration of the treatments showed an obvious increase from the beginning to the 48th hour. It is determined that an irregular fluctuation is present at the 72nd hour. It was seen that there was a decrease-increase-decrease tendency in the data obtained at the 72nd hour. When the elimination values were taken into account, the 5-ppm concentration showed the highest survival rate, while the data obtained from the other treatment groups showed a tendency to increase from the control group and then to decrease thereafter. It seems that the results are opposite to those expected from these values and the quantum dots show different characteristics in the saline environment. PbSe quantum dots, which are toxic even at low ratios, did not show toxic effects at high concentrations in the marine environment. Data obtained were below the losses expected for the organisms.

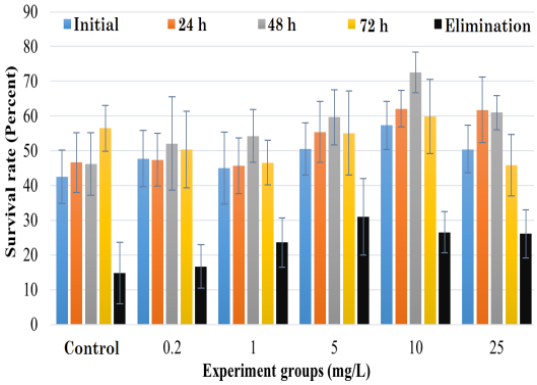


Fig. 9. The survival rates of Artemia salina exposed to PbSe quantum dots.

4. Conclusions

Every research made to investigate the use of nano-structured materials in biotechnology and bioengineering, and their effects, which are directly related to human health is very important. The synthesis of quantum dots and their encapsulation with suitable encapsulants will increase the potential use of these dots in future years. The coating of nano-sized structures or quantum dots with materials such as biopolymers not only reduces toxic properties, but also improves structural stability, size and morphology. In this context, PbSe quantum dots obtained by chemical methods in laboratory environment are covered with chitosan which is a natural biopolymer and used in many industrial branches. Chitosan is not biologically toxic, it is a substance that increases the biocompatibility of dangerous nanocrystals.

When we examine the morphological results of all the PbSe quantum dots obtained by chemical synthesis, it is seen that the majority of them display round or spherical structure. There is a homogenous range between 2-15 nm for the sizes of these and aggregation amount of them is very low. It seems that there is a growth of a few or 10 to 100 folds in the size distribution of these particles. This is a general characteristic of nano-sized particles and it is observed that they are growing in liquid medium. It is observed that they are positive (+) in terms of surface load and all the quantum dots are very stable. In addition, the increase in oxidation resistance of PbSe coated with chitosan was found in XRD results. In the XRD pattern of the uncoated sample, PbSeO3

oxide peaks were found to be significant. In the quantum dots coated with chitosan, no peaks pointing to oxidation have been observed. It can be said with ease that the coating applied improves the oxidative resistance. It is understood from the SEM images that although chitosan coated PbSe's have higher rate of aggregation, they are more homogenous in morphological terms and have a spherical structure. As a result of bioassay with the PbSe obtained in laboratory environment, it is seen that aquatic organisms are taking PbSe present in the medium. The quantum dots show different characteristics in the saline environment. PbSe quantum dots, which are toxic even in low concentrations in fresh water, did not show toxic effects at high concentrations in the marine environment. Data obtained were below the losses expected for the organisms.

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