Synthesis of CdSe Nanoparticles and Their Effect on the Antioxidant Activity of Spirulina Platensis and Porphyridium Cruentum Cells

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***Abstract* – Single-crystalline cadmium selenide nanoparticles were obtained using HTSPS synthesis. X-Ray powder diffraction and transmission electron microscopy were used to confirm the crystallinity and morphology of the resulting nanoparticles. To study the action of CdSe on antioxidant activity, we selected two biotechnological important strains of microalgae: cyanobacteria *Spirulina platensis* and red microalga *Porphyridium cruentum*. In the case of *Porphyridium cruentum*, the obtained results demonstrated an increase in the productivity. For *Spirulina platensis*, the presence of the compound in the cultivating medium decreased the productivity of cyanobacteria.**

***Keywords* - CdSe nanoparticles, HTSPS synthesis, antioxidant activity, colloidal solutions**

I. INTRODUCTION

Nanomaterials are considered promising modifiers of surface structures of cells. For example, well known polymers, microparticles, nanoparticles, and their possible combinations are widely used in these scopes. Several papers described the immobilization of nanoparticles on the surface of various cells. In particular, they showed the deposition of gold nanoparticles on the surface of *E. coli* cells in order to form electrical microcontacts. Complexes of nickel nanoparticles and the bacterial cells were obtained and characterized in order to design magnetic microdevices on their basis. The surface of yeast cells *Kluveromyces fragilis* was modified with magnetic nanoparticles, resulting in effective sorbents, which were obtained on the basis of magnetized cells. Thus, the study of the interaction of nanomaterials with living cells is of particular interest and is caused by the fact that hybrid systems based on nanomaterials and living cells can be prepared and used to identify the toxic properties of nanomaterials, which is aimed at changing the properties of cells, regulating their physiological activity, and visualizing cellular organelles as well as for high-precision spectral identification of living cells based on differences in biochemical composition of their surface structures [1]. In this connection, it is extremely urgent to find methods of the immobilization of nanomaterials on cell surfaces that will allow them to maintain physiological activity.

II. METHODS

Chemicals**.** Tri-*n*-octylphosphine (Aldrich, 90%), amorphous selenium shot (Aldrich, 99.999%), squalane (Aldrich, 99%), diphenyl ether (DPE) (Fluka, 98%), cadmium acetate dihydrate (Aldrich, 99.99%), and oleic acid (cis-9-octadecenoic acid, Aldrich, 90%) were used as purchased without further purification. Anhydrous ethanol, hexane, chloroform, acetone, tetrachloroethylene, and trichloroethylene were purchased from different companies and used without further purification. Trioctylphosphine telluride (0.75 M, TOP-Se) was prepared by the complete dissolving of the necessary amount of tellurium in 50 ml of TOP at 60–70°C under moderate stirring. The TOP-Se solution described above was prepared and stored in a nitrogen glove box.

The microalgae cultivating and obtaining of extracts from biomass. The strains of *Porphyridium cruentum* and *Spirulina platensis* were cultivated under laboratory conditions. Water extracts were prepared from native biomass by freezing, and ethanol extracts were derived with 70% ethanol in ratio 1/10 (w/v).

Synthesis. The HTSP method was used as the basis for preparing cadmium selenide nanoparticles. A standard synthesis of CdSe nanoparticles was performed in a round-bottom three-neck flask equipped with a magnetic stirrer, a thermocouple, and a temperature control unit. Cadmium oleate was prepared by heating a mixture of 2 mmol cadmium acetate, 4 mmol of oleic acid, and 20 mmol of squalane or 20–25 ml of diphenyl ether. Oleic acid was employed both for group II precursor formation and nanoparticle stabilization during the synthesis intended for nucleation and reaction rate control. This solution was heated under vacuum at 75–80°C for 5–6 h in order to form cadmium oleate and remove already formed acetic acid. The subsequent synthesis of cadmium selenide NPs was carried out by rapid injection of trioctylphosphine selenide (TOP-Se) solution maintained at room-temperature into a vigorously stirred mixture containing cadmium oleate heated from 140 to 200°C under N_2 atmosphere. The reaction mixture was maintained at a fixed temperature for 10 min and then promptly cooled to room temperature using an ice-water bath. The solution quickly turned dark red during the synthesis due to the formation of CdSe colloidal solution. The TOP-Te/lead oleate molar ratios varied from (1.5–3) to 1.

A solvent containing two parts of hexane, one part of anhydrous ethanol, and five parts of acetone was prepared to purify the nanoparticles from unreacted precursor, excess

surfactant, and high-boiling point solvents. A size-selective precipitation was carried out by centrifugation, using a polar/nonpolar solvent combination, consisting of acetone and either hexane or chloroform. After precipitation, the CdSe nanoparticles were isolated and re-suspended in chloroform, hexane, and trichloroethylene followed by ultrasonic treatment to form stable colloidal solutions used for further preparation and characterization. The chemical analysis and atomic absorption spectroscopy confirmed the CdSe composition of the nanomaterial deposited after multiple purifying and re-suspension of the original solution.

In order to use hydrophobic nanoparticles for biological applications, they first must be transferred into aqueous solution.

The formation of various surface-active functional groups (-COOH, -C=O,-OH) makes it possible to transfer to aqueous colloids and contributes to coordination interaction with necessary surfaces, for example, with biological molecules [2].

Methods for the modification of CdSe nanoparticles include the processing of nanoparticles with buffer solutions, transferring into a soluble state, and obtaining a colloidal solution in the presence of modifying agents such as 1-thioglycerol. The subsequent deposition and re-dispersion of nanoparticles was performed in deionized water [3].

Antioxidant activity by the $ABTS^+$ radical cation assay. The total antioxidant activity of extracts was measured by the $ABTS^+$ (2,2 azinobis 3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization [4]. $ABTS^+$ was generated by the oxidation of ABTS (7mM) with potassium persulphate (2.45 mM). The reaction mixture was left at room temperature overnight (12-16 h) in the dark before use. Prior to tests, the $ABTS^+$ stock solution was diluted to an absorbance of 0.700 ± 0.020 at 734 nm. Then 1 ml of diluted $ABTS^+$ solution was mixed with 10 µl of the test sample, and the absorbance was measured after 6 min.

Nanocrystalline sample characterization. High-resolution transmission electron microscopy (HRTEM), powder X-ray diffraction (XRD), and infrared absorption spectroscopy (IR) were used to characterize the size, shape, structure, and composition of the CdSe nanocrystals and the optical properties of the capping layer. The powder XRD data were recorded with CuKα radiation (λ = 1.5406 Å) using Scintag and PANanalytical X'Pert Pro diffractometers, both operating in the Bragg–Brentano geometry. Samples for the XRD measurements were prepared by the deposition of concentrated CdSe colloidal solutions in chloroform or trichloroethylene onto a glass substrate. The 2θ range scanned from 20° to 80°. A Philips CM 30 transmission electron microscope (TEM) equipped with a Super-Twin lens and LaB_6 emitter was used for HRTEM measurements. All the images were taken at 300-kV accelerating voltage and recorded with a megapixel CCD camera. The EDX spectra were collected using a Tecnai F30 TEM operating at an accelerated voltage of 300 kV and equipped with a Schottky field emission electron source and a Super-Twin lens. Samples for the TEM were prepared by the deposition of a drop of a dilute colloidal solution in chloroform, hexane, or trichloroethylene on a carbon-coated copper grid (200 mesh), allowing slow evaporation at room temperature. The IR absorption spectra were recorded with a VERTEX-70 Fourier transform spectrometer (Bruker Corp.). Each spectrum was obtained at room temperature by averaging over 64 interferograms with a resolution of 1 cm^{-1}. The samples for the IR measurements were prepared as pellets with KBr or CsI powders. The quantitative analysis of the resulting nanopowders was performed with an AAS-3 atomic absorption spectrometer using acetylene–air flame. For the investigation of the resulting nanomaterial, the following synthesis parameters were selected: fixed reaction temperature T = 175°C, TOPSe/cadmium oleate molar ratio r = 2.0. Diphenyl ether was used as the high-boiling heat-transfer agent. As mentioned above, the samples were prepared for X-ray powder diffraction by depositing the colloidal solution onto a glass substrate dropwise.

III. RESULTS AND DISCUSSION

The typical powder diffraction patterns of resulting CdSe nanocrystals are shown in Fig.1. XRD revealed three broad peaks positioned at 2θ = 25.37, 42.04, and 49.63° with corresponding interplanar spacings of 3.51, 2.15, and 1.83 Å, respectively. These peaks are uniquely assigned to the (002), (110), and (112) planes of the wurtzite structure of CdSe. The broadening of the diffraction pattern for CdSe implies a reduction in particle size.

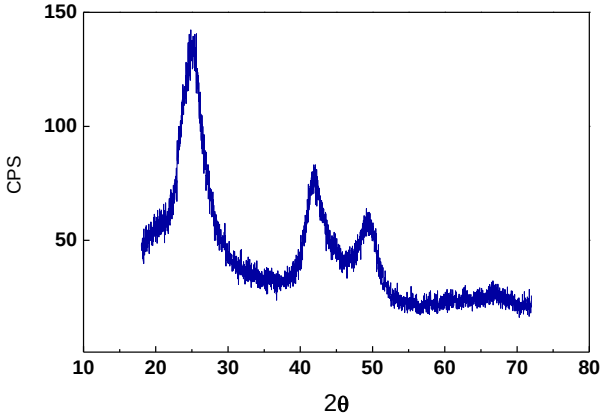

Fig. 1. Typical powder diffraction pattern of CdSe NPs.

An average size of 4–4.5 nm for the CdSe nanoparticles was estimated from the X-ray powder diffraction data using the Scherrer equation.

The size and shape of cadmium selenide nanoparticles were examined using TEM methods.

To study the action of CdSe on antioxidant activity, we selected two biotechnological important strains of microalgae: cyanobacteria *Spirulina platensis* and red microalga *Porphyridium cruentum*. The previous studies showed the increased adaptability of strains to changing cultivating conditions. The adaptation response in the majority of cases is considered to be the response to the stress factor, which is manifested in different modes at the mentioned strains depending on specific functional and constitutive structures [5].

One of the essential markers of adaptability is strain productivity. The CdSe nanoparticles were supplemented in the cultivating medium on the first day of life cycle to verify the compound's toxicity level. In the case of *Porphyridium cruentum*, the obtained results demonstrated the increase in productivity by 34-47.5% (CdSe concentration was 4.0-6.0 mg/l) and by 18% in the case of the highest concentration of 8.0 mg/l. For *Spirulina platensis*, the presence of the compound in the cultivating medium decreased cyanobacteria productivity by 33% in the case of CdSe

concentration of 4.0 mg/l. Higher concentrations are fatal; the culture dies on the 3rd day of cultivation.

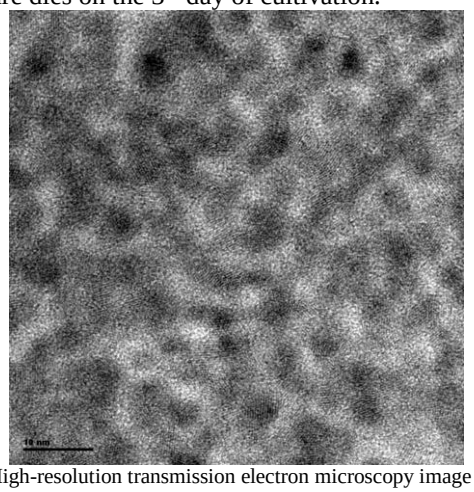

Fig. 2. High-resolution transmission electron microscopy image of small aspect ratio CdSe NPs.

Another index of culture adaptation to the cultivating conditions is the strain's antioxidant activity. Cyanobacteria and microalgae synthesize the complex of substances with antioxidant and antiradical properties [6]. Depending on factors which induce radical accumulation, the synthesis of the necessary antioxidant compounds with the modification of antioxidative statute took place. This fact could be determined by the antioxidant activity tests of extracts from biomass. Two types of extracts were prepared: water and ethanol extracts. We used the antioxidative test with ABTS radical.

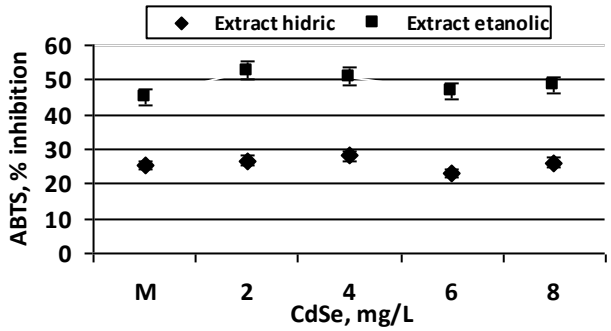

Fig. 3. The antioxidant activity (% of ABTS inhibition) of extracts from *Porphyridium cruentum* biomass.

The analyses of the results obtained for *Porphyridium cruentum* biomass show the relative stability of antioxidant activity for the two types of extracts (Fig. 3). In the case of supplementing CdSe in the cultivating medium in tested concentrations, the antioxidant activity of the water and ethanol extracts oscillated identically to the control sample, without excess of accumulating antioxidants. On the other hand, the presence of CdSe nanoparticles in the cultivating medium has no essential influence on the maintenance of antioxidant and antiradical compounds in *Porphyridium* biomass, inducing an increase in productivity.

In the case of *Spirulina platensis* extracts, an increase in the antioxidant activity was revealed (Fig. 4). So, for the extracts obtained from the biomass cultivated with a supplement of 2.0 mg/l CdSe, the antioxidant activity of the ethanol extract increased by 25%, but the water extract showed the same result as the control sample. The increase in the CdSe concentration up to 4.0 mg/l induces an increase in the antioxidant activity by 70-73% for both types of extracts.

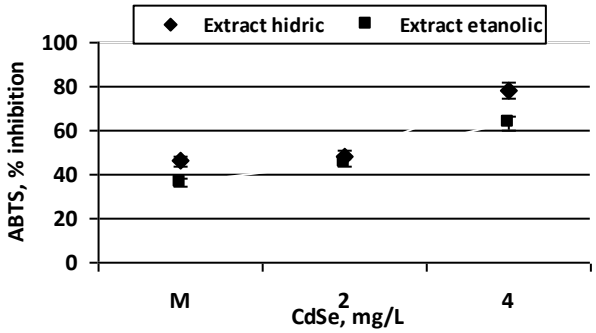

Fig. 4. The antioxidant activity (% of ABTS inhibition) of extracts from *Spirulina platensis*.

For cyanobacteria *Spirulina platensis* strain, the presence of CdSe particles in the cultivating medium is a strong factor for stimulating antioxidant compound synthesis; the limitation of its increase is a concentration of 4.0 mg/l.

IV. CONCLUSIONS

Cadmium selenide nanoparticles were obtained; their composition and crystallinity were confirmed. The CdSe nanoparticles were supplemented in the cultivating medium (cyanobacteria *Spirulina platensis* and red microalga *Porphyridium cruentum*), and the change in the productivity was studied.

The obtained results suggest the idea of different nature of adaptation mechanisms in prokaryote and eukaryote organisms. The confirmation of this assumption on the basis of other phycologic objects will offer the possibility to develop models for testing the nanoparticle toxicity.

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