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Properties and biocompatibility of colloid cadmium sulfide nanoparticles

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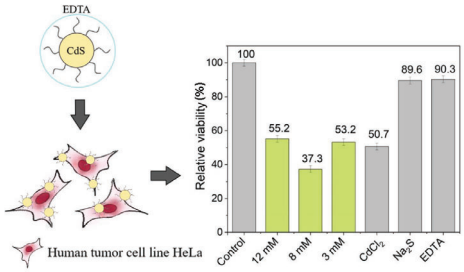
The properties and cytotoxicity of aqueous colloidal solutions of cadmium sulfide CdS nanoparticles obtained by chemical condensation were explored. Dynamic light scattering and optical spectroscopy were used to study the hydrodynamic diameter, size distribution, zeta-potential and optical properties of the CdS nanoparticles in solution. The cytotoxicity of colloidal CdS nanoparticles at different concentrations was assessed on cultures of human dermal fibroblasts and the cancer HeLa line.



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Cadmium sulfide CdS nanostructures attract the attention of researchers due to their optical properties. Their intense and effective tunable luminescence and photostability make them applicable in optoelectronics,[1] biology and medicine.[2] The luminescent nanoparticles stabilized in an aqueous colloidal solution are widely used as fluorescent labels to visualize both cell structures and the molecules involved in their metabolism. This application demands a deep understanding of not only the physical properties, but the cytotoxicity of a solvent and nanoparticles as well.[3–5] Additionally, the bioimaging sets a number of requirements to the labels based on semiconductor nanoparticles such as water solubility, biocompatibility, and storage stability. The water-soluble nanoparticles are commonly synthesized in organic solvents followed by solubilization. This technique requires complex additional procedures to obtain hydrophilic nanoparticles. Thereby, the development of methods for the synthesis of stable nanoparticles directly in a water solution is important. The main challenges are to keep the aggregative stability and control the size and size distribution due to its high effect on the luminescence. Moreover, the biocompatibility depends on the concentration, size and zeta potential of the nanoparticles.[4–6] Positively charged nanoparticles with a diameter of 8 nm are toxic to cells, whereas those with a diameter of about 30 nm can be effective against cancer cells. Previously, the toxic influence of the CdS nanoparticles concentration on the interaction with various cell cultures was studied by fluorescence microscopy.[7] However, the study of the cytotoxicity of CdS solutions using the methyl thiazolyl tetrazolium (MTT) test has not been carried out yet.

The aim of this research was to study the cytotoxicity of colloidal CdS nanoparticles at different concentrations using standard MTT assay. Additionally, the toxic effect of initial solutions used in the synthesis of nanoparticles was investigated since the samples contain residues of the precursors.

Cadmium sulfide nanoparticles in an aqueous solution were synthesized by chemical condensation[9–11] based on the exchange reaction.[†] Disodium salt of ethylenediaminetetraacetic acid (EDTA) was used as a stabilizer to prevent agglomeration and sedimentation of the nanoparticles.[12] This chelating agent[13] provides long-term stability of the resulting colloidal solutions. The negative charge of EDTA anions may play an important role in the interaction between particles and cell. It was found that high positive values of the zeta potential of Ag_2S nanoparticles, when interacting with blood cells, lead to an effect similar to blood clotting.[14] Additionally, the effect of CdS concentration on a luminescence intensity and visualization of cells during optical microscopy investigation was studied before.[7,10,11] As a result, the concentration interval providing the bright luminescence of CdS was established. Therefore, the solutions with molar concentration of CdS nanoparticles equal to 3, 8 and 12 mM were synthesized to study the effect of CdS concentration on the stability and biocompatibility. The hydrodynamic diameter (D_H), size distribution (standard deviation) and zeta-potential (ζ) of CdS nanoparticles in solution herein found are presented in Table 1.

The colloidal solutions remain stable for more than 6 months. The average D_H of CdS nanoparticle was about 13 nm and did not depend on the concentration of the dispersed phase or time.

[†] Solutions of initial reagents, $CdCl_2$, Na_2S and EDTA, were preliminarily prepared, and the chelating agent solution was mixed with the $CdCl_2$ solution, with the $CdCl_2$/EDTA molar ratio being 1 : 1. The equimolar amount of the resulting mixture was then added to the Na_2S solution. The premixing of the EDTA solution and $CdCl_2$ is associated with the results of previous experiments. Preliminary mixing of an EDTA solution with a $CdCl_2$ solution leads to the formation of complexes, which subsequently affects the rate of formation of CdS and the stabilization of the resulting colloidal solution. Thus, this sequence ensures the gradual formation of a dispersed phase and avoids coagulation of CdS nanoparticles.

Table 1 Hydrodynamic diameter and zeta-potential of CdS nanoparticles.[a]

Sample	*C*(CdS), mM	D_H[b]/nm	ζ[c]/mV
CdS-1	12	13±5	–25±9
CdS-2	8	14±5	–25±9
CdS-3	3	13±4	–28±7

[a] Hydrodynamic diameter and size distribution were measured by dynamic light scattering at 25 °C on the Zetasizer Nano ZS (Malvern Instruments Ltd.). Zetasizer Nano ZS is equipped by a 4 mW He-Ne laser operating at a wavelength of 633 nm. The scattered light was detected at angles of 173°. All measurements were repeated 3 times for better statistics. The measurement of zeta-potential of particles in the Zetasizer Nano ZS was made using the technique of laser Doppler electrophoresis. [b] With standard deviation. [c] With measurement error.

The constant values of D_H and high ζ (in the error limits) show the satisfactory stability of the micelles formed by CdS core, EDTA adsorbed layer and solvate shell. Attractive van der Waals energy between particles dispersed in a liquid medium depends on particles concentration and distance between them. Thus, at some limit, the concentration of nanoparticles leads to shortening of interparticle distance, increasing of attractive forces and formation of large agglomerates. However, at the selected concentrations, this limit was not reached which leads to aggregative and sedimentation stability of colloids.

The optical absorption and luminescence spectra are shown in Figure 1. The synthesized CdS nanoparticles provide a broad luminescence band, which is attributed to the dominance donor–acceptor pair transitions due to the presence of defects in the atomic structure (trap states).[15,16] Nevertheless, the observed emission of CdS is located in the visible and near-infrared region, which allows one to study the cells using optical microscopy under UV excitation. The values of the band gap energy (E_g) of CdS nanoparticles were calculated from the optical absorption. The E_g value remains constant and equal to 2.60 eV regardless of the CdS concentration. This value exceeds the E_g of the bulk CdS material (2.42 eV) that indicates the existence of a quantum size effect. The quantization of electron levels shows up as a blue shift of the absorption edge and luminescence maximum. Thus, the synthesis of CdS by chemical condensation directly in an aqueous medium leads to the formation of nanoparticles with an average diameter of about 6 nm calculated from the band gap broadening due to the quantum size effect. The increase in optical absorption and luminescence intensity while the E_g and position of luminescence remains unchanged indicates the increase in the particle quantity instead of their enlargement. Moreover, it proves the stability of nanoparticles in the interval of studied concentration.

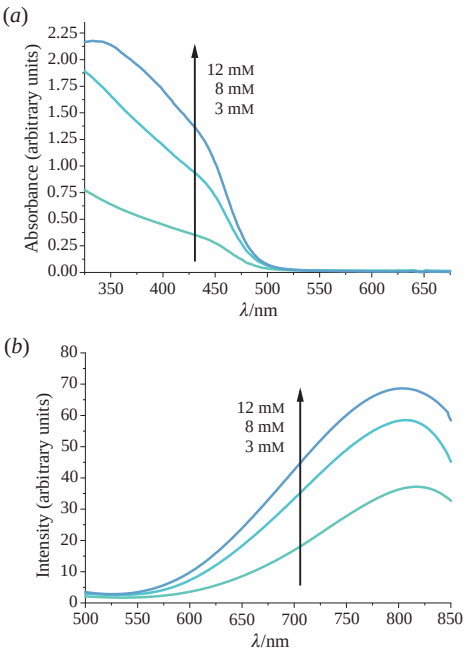

Figure 1 The effect of CdS concentration in the solution on the (*a*) optical absorption and (*b*) and luminescence (FS-5 spectrofluorometer, Edinburgh Instruments, UV, visible and NIR ranges, ambient temperature, excitation at λ = 465 nm).

To study the cytotoxicity of the nanoparticles, human dermal fibroblasts and cancer HeLa cells were incubated with CdS colloidal solutions of various concentrations for 72 h. Additionally, the cells were incubated with initial solutions of $CdCl_2$, Na_2S, and EDTA (6 mM) for 72 h to study the toxic effect of precursors. This was necessary as the filtration step was not used in the synthesis of nanoparticles. The cell viability was then assessed using a standard MTT assay.‡ This is a simple way to evaluate cell survival, cytotoxic effect and proliferation. The method is based on the ability of living and metabolically active cells to absorb the yellow soluble tetrazolium salt and reduce it to purple insoluble formazan crystals.

All tested samples affected the both cell cultures (Figure 2). Based on the numerous studies, the nanoparticle toxicity may be due to either some inherent chemical feature or their nanoscale properties. Aspects related to inherent toxicity are mostly due to the elements contained in the nanoparticle core. Elemental toxicity is considerably dependent upon the accessibility of the core atoms to the surrounding solvent. The following criteria were used to evaluate the degree of cytotoxicity of nanoparticle solutions in relation to cell cultures: the cytotoxicity index (IC) of more than 70% indicates the high cytotoxicity; IC of 40–70%

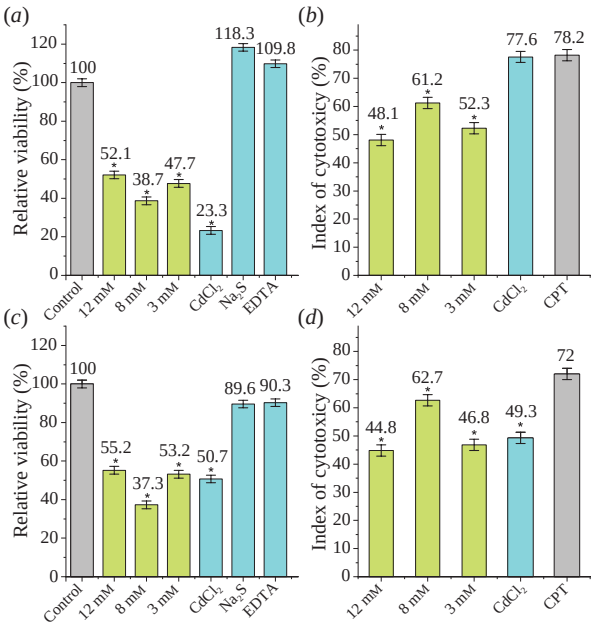

Figure 2 The effect of CdS nanoparticles concentration (green bars) and initial salt solutions (blue bars) on the viability of (*a*),(*b*) human fibroblasts and (*c*),(*d*) HeLa: (*a*),(*c*) relative viability of cells and (*b*),(*d*) cytotoxicity index. A symbol (*) indicates reliable distinction from the cell cultures (control) and camptothecin (CPT), $p \leqslant 0.05$.

‡ The MTT test was performed in triplicate with negative (a cell culture) and positive (a solution of the cytotoxic drug camptothecin at a concentration of 3 mmol dm^{-3}) reference samples. The optical density of the formazan solution in the experimental and control wells was determined on an automatic spectrophotometric flatbed scanner Tecan Infinite M200 PRO at a wavelength of 570 nm. The cytotoxicity index (IC) was calculated using the formula $IC = (K - O)/K \times 100\%$, where K is the optical density in control samples, O is the optical density in experimental samples.

relates to the moderate cytotoxicity, IC less than 40% specifies the low cytotoxicity. The different biological activity was detected with respect to the fibroblasts [see Figure 2(*a*),(*b*)] and HeLa [Figure 2(*c*),(*d*)]. The IC of samples that contained CdS nanoparticles indicated the moderate cytotoxicity in relation to fibroblasts [Figure 2(*b*)]. The solution of $CdCl_2$ exhibited the same high cytotoxicity for fibroblasts as camptothecin that is associated with the toxicity of free Cd^{2+} ions dissolved in the probe.[17] However, the IC of solutions of CdS nanoparticles and precursors with respect to the HeLa line failed to reach the values of the camptothecin [Figure 2(*d*)]. This indicates the insufficient cytotoxicity of these solutions with respect to tumour cells. The reasons of low biocompatibility and high cytotoxicity of the sample with moderate concentration (8 mM) are unclear and require further investigation. The similar difficulty to compare toxicity based on particle concentrations was also observed in other experiments with CdSe/CdS nanoparticles.[18] More interestingly, the ICs of samples CdS-3 (see Table 1) and $CdCl_2$ with respect to the cancer cells are similar. However, the concentration of Cd in sample CdS-3 is twice less than in the sample of $CdCl_2$. Moreover, the most part of cadmium is bind to sulfur in the non-soluble sulfide (at pH > 4). Taking into account this observation, we supposed that EDTA capped CdS toxicity may be a function of cell ingestion/uptake and not only due to possible leaching of ions from the nanoparticle to the cell environment.[17,19]

The nanoparticle toxicity variation with different cell lines was reported previously in a number of studies as well.[17,19] The two possible reasons for this were suggested. First, the different uptake rate of nanoparticles between the different cell types might be the driving force that causes the variation of toxicity. Second, the differences of the cell viability in the two cell lines may come from the variation of cell sensitivity towards oxidative stress, which is highly related to the expression level of the redox protein thioredoxin. The thioredoxin acts as a growth factor and has elevated content in many human primary cancers including gastric carcinoma when compared to a normal tissue. Upon analyzing these results, the property of specific cell type, such as nanoparticle uptake efficiency, cell membrane surface property and protein and hormone expression of the cell, may play a key role in the level evaluation of nanoparticle toxicity. Thus, it is necessary to develop and standardize a comprehensive set of assessment schemes for the proper investigation of nanoparticle toxicity.

In conclusion, the effect of CdS concentration on the average size, size distribution, colloidal stability in the aqueous medium, luminescence and biocompatibility was explored. Negatively charged EDTA was used to synthesize and stabilize the CdS nanoparticles directly in water. The colloidal solutions of CdS nanoparticles in all concentrations studied showed a moderate level of cytotoxicity with respect to the culture of human dermal fibroblasts. Filtration and dialysis of the sample are required before the incubation with cells to decrease the cytotoxic effect of CdS nanoparticles. Additionally, the results proved that the toxicity of nanoparticles does not solely depend on a single factor but rather depends on a combination of elements from the particle composition and extent of cellular uptake. This requires further study of changes in the cellular processes across the cell lines.

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