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Synthesis and assessment of antibacterial effects of CdSe:Ag nanoparticles produced by chemical precipitation method

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ABSTRACT: Chemical precipitation method was used in order to synthesize CdSe:Ag quantum dots (2-3 nm). Their Physical properties and characteristics were assessed by X-ray diffraction, ultra violet-visible spectrophotometer and TEM (Transmission Electron Microscope) and it was shown that the obtained CdSe:Ag quantum dots are cubic with high-quality. Antibacterial effects of CdSe:Ag nanoparticles against some pathogen bacteria were investigated. *Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella typhi* were used as test microorganisms. Disc bacteriological tests were used in order to assess the effects of CdSe:Ag concentrations 13.4-1.05 mg/ml as antibacterial agent. And the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericial Concentration) of CdSe:Ag nanoparticles which are required to inhibit the growth of these three bacteria is determined by applying Broth Dilution Method. According to the results, by increasing the concentration of CdSe:Ag the inhibiting effect rises. The MIC values to inhibit the bacteria *Pseudomonas aeruginosa, Staphylococci aureus* and *Salmonella typhi* are 4.2, 3.35 and 6.7 and the MBC values to do so are 13.4, 8.4 and 13.4, respectively. In conclusion, by increasing the nanoparticle concentration in wells and discs, the growth inhibition and diameter of inhibition zone has also been increased.

Keywords: *Antibacterial effect, Pseudomonas aeruginosa, Quantum dots, Staphylococci aureus, Salmonella typhi*

INTRODUCTION

Colloidal semiconductor nano-crystals generally consist of II-VI or III-V groups of elements table named quantum dots (QDs). Similar to other nanoparticles, QDs optical and electrical properties assessment show that their characteristics are strongly size dependent (Huang, *et al.*, 2013, Okasha, *et al.*, 2010). They have many useful applications in different areas like industry and science. On the other hand, most chemicals used for their production are toxic, expensive, and even explosive (Amiri, *et al.*, 2010, Amiri, *et al.*, 2011).

Cadmium selenide (CdSe) is one of the most interesting QDs with dark red appearance. It has two different structures as solid hexagonal or cubic crystal. CdSe:Ag is an n-type semiconductor with a band gap of 1.74 V at room temperature. Its molecular weight is 191.37g/mol. The percentage of Cd in that is 58.74% and that of Se is 41.26%. CdSe:Ag nanoparticles have some characteristics which are completely different from bulk form. Researchers are concentrating on developing controlled synthesis of CdSe:Ag nanoparticles (Amiri, *et al.*, 2013).

There are many methods of synthesizing CdSe nanoparticles such as photochemical route, wetted

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chemical method, low-temperature hydrothermal method and solvothermal process (Met, 2012). A large surface-volume ratio of nanoparticles in comparison with bulk one, elevates the range of probable interaction with cell surface (Hosein Zade, *et al.*, 2012) The antibacterial effect of nanoparticles such as ZnO, MgO, TiO_2, SiO_2 is considerable and therefore their selective toxicity towards biological systems suggests their potential application as therapeutics, diagnostics, surgical devices and Nano medicine based antimicrobial agents (Amiri, *et al.*, 2014).

The action mechanism of silver against the bacteria has not been fully comprehended yet. The silver particles probably attach the cell membrane and as a result, the vital activities of that cell such as respiration and nutrition intake are stopped. By increasing the concentration of the particles the permeability and antibacterial properties rise. In fact the Ag particles bond with the existing Phosphorus and sulfur compounds in DNA and kill the germs (Song, *et al.*, 2008)

In this study, it is tried to assess the CdSe:Ag QDs antimicrobial effect on the three pathogen bacteria (*Pseudomonas aeroginosa, Staphylococcus aureus, Salmonella typhi*). The inhibition effect of different CdSe:Ag concentrations on the applied area is also studied.

MATERIALS AND METHODS

Synthesis and assessment of properties

Chemical precipitation method was used in order to synthesize CdSe:Ag nanoparticles. At first, three solutions of Cadmium Chloride ($CdCl_2.4H_2O$), Mercapto-ethanol (ME) Sodium Selenide ($Na_2SeO_3.5H_2O$) and silver nitrate ($AgNO_3$) (all from Merck Company, Table 1) were prepared in the distilled water, under vigorous stirring. After that, Cadmium Chloride solution was poured into a three spout balloon container. Then, silver nitrate solution and after that mercapto-ethanol solution were added to the same balloon, it should be noted that one droplet of the solution is added every 3 seconds. Finally, Na_2SeO_3 was added to the balloon following the same way. In order to extract any impurity, the final solution was washed by deionized water several times and then was centrifuged. Finally, the precipitated sample was dried. All processes were done at room temperature (Amiri , *et al.*, 2013).

XRD (X-Ray Diffraction, Bruker D8 ADVANCE λ= 0.154nm Cu Kα radiation) and UV-Vis spectrophotometer (Ultra Violet–Visible, UV-2600 Shimadzu, Japan) were used to investigate CdSe QDs optical and structural properties. Particle size distribution was assessed by TEM (Transmission Electron Microscope, Philips CM 10 HT-100).

Antibacterial Activity Assay

The antibacterial effect against *Pseudomonas aeroginosa, Staphylococcus aureus* and *Salmonella Typhi* were studied by the culture method. They were grown aerobically in nutrient broth culture environment for 24 h at 37°C before using as target organisms. Then 1 CC of each bacterium was planted to a plate. The plates were previously prepared by Mueller Hinton Agar culture environment. After that, a well was created in the plates by the Pasteur pipette.

From this bacteria–rich suspension, a suspension with 0.5 McFarland concentrations is prepared. It is then moved to a MHA and cultured in three directions with sterile swap. After that some wells with the diameter of 6 mm and 20 mm from each other are created. Using the Sterile Swap some of the suspension is taken and cultured in different directions. CdSe:Ag nanoparticles with different concentrations (13.4, 8.4,

Table 1: Details of the used materials for synthesizing CdSe:Ag nanoparticles.

Material name	Purity (%)	Used mass or volume
$AgNO_3$	99%	0.085 gr
$CdCl_2.2.5\ H_2O$	99%	2.28 gr
$Na_2SeO_3.5\ H_2O$	99%	2.63 gr
C_2H_5OSH	99%	400 µl

6.7, 4.2, 3.3, 2.1, 1.65, 1.05) were prepared and applied. Rich bacteria were prepared with the aid of Luria Bertani Broth culture and then different mentioned concentrations of CdSe:Ag were applied on these cultures, one of them lacked nanoparticles was considered as the control pipe; the pipes were all covered by sterile cotton. The pipes were incubated at 37°C for 24 hours. To determine MIC, 100 microliters of the suspension removed and transferred on Mueller Hinton agar culture and spread on it using sterile swap; they were incubated for 24 hours. The tube of low concentrations of nanoparticles in which no turbidity was caused by the growth of bacteria is considered as MIC. And the culture in which 99.9% of the bacteria were killed and only 0.1% of them had grown is considered as MBC. To minimize the errors, the examination was repeated 3 times.

RESULTS AND DISCUSSION

Fig. 1. shows the XRD pattern of the CdSe:Ag QDs. As can be seen, the sample has the cubic crystal structure and also is single phase. These findings are according to the standard JCPDS (Joint Committee on Powder Diffraction Standards) card No. 19-0191 and the diffraction peaks correspond to the (111), (220) and (311) are the crystal plane. The mean size of the particles was calculated 1-2 nm by Debye-Scherer formula.

The TEM photo of the sample is shown in Fig. 2. The size was determined around 2-3 nm from TEM photograph. Generally, the electronic state is an important property of semiconductor; it is described in terms of conductivity bands and valence and a band gap between them. However, the wavelength of the electrons is closer to the range of the particle sizes as the particles size becomes lower. It means the laws of classical physics have to be substituted by quantum confinement or quantum size effect (QSE). Moreover, many studies have reported the QSE in direct-gap semiconductors such as a shift of the optical absorption edge to higher energies with decreasing size (Lamia, *et al.*, 2015).

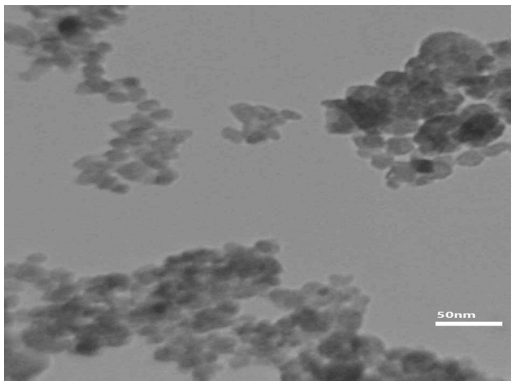

Fig. 2. Transmission Electron Microscope of CdSe:Ag QDs

The absorption spectra of CdSe:Ag QDs at are presented in Fig. 3. The UV-Vis absorption spectrum in 37 °C showed that the absorption peak of the obtained CdSe:Ag QDs, is 380 nm (3.25eV) whereas it is 698 nm (1.78eV) for bulk cubic CdSe:Ag (Amiri , *et al.*, 2013).

The size can also be estimated by Bruce equation, as being (2- 3 nm) (Park, *et al.*, 2012).

$$\Delta E_g = E_g^{QD} - E_g^{Bulk} = \frac{\pi^2 h^2}{2MR^2}$$

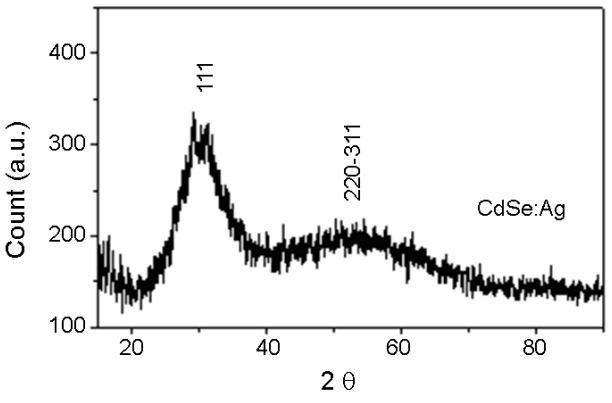

Fig. 1. XRD patterns of the CdSe:Ag QDs

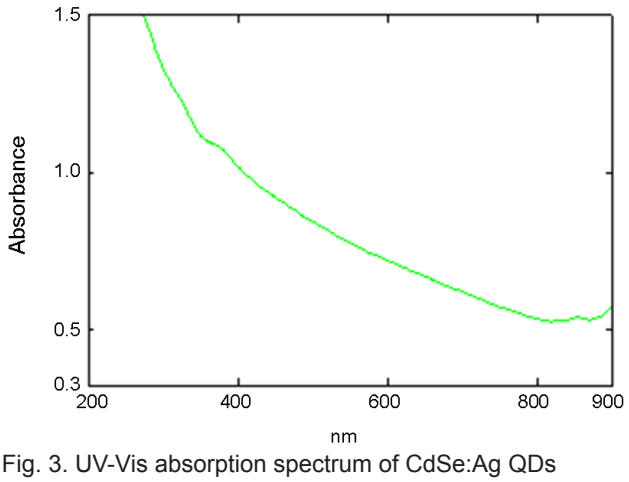

Fig. 3. UV-Vis absorption spectrum of CdSe:Ag QDs

S. Typhi *P. aeroginosa* *S. aureus*

Fig. 4. Antibacterial activity of CdSe:Ag nanoparticles on *S. aureus, P. aeroginosa* and *S. typhi*

Where E^{QD}_{g} and E^{Bulk}_{g} are the energy gap of nanoparticle and bulk respectively, h is the Plank constant, R is the nanoparticle radius and M is the reduced mass, me is the electron mass and mh is the hole mass.

$$\text{Reduced mass} = \left(\frac{1}{m_e} + \frac{1}{m_h} \right)$$

Therefore, CdSe:Ag band gap changes depending on the nanoparticle size. Our findings are completely compatible with previous studies (Amiri, *et al.*, 2013). The antibacterial activity of CdSe:Ag QDs was estimated by the disc and well diffusion agar methods and the results are shown in Table 2, Figs. 4 and 5. The size of the inhibition zone indicated the antibacterial effect of CdSe:Ag QDs (Hosein Zade, *et al.*, 2012). As can be seen, by increasing the CdSe concentration in wells and discs, the growth inhibition has also been increased.

The size of inhibition zone was different according to the type of bacteria and the concentrations of CdSe:Ag QDs.

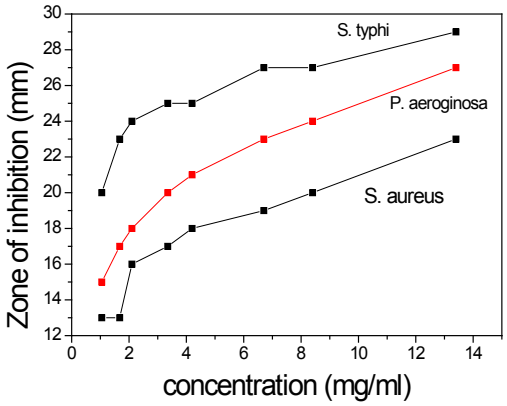

Fig. 5. Comparison of zone of inhibition for different concentration of CdSe:Ag quantum dots

Based on the results obtained from Fig. 4. and the diameter of inhibition zone for different bacteria, it can be concluded that the maximum inhibition activity happens for *Staphylococcus aureus* in comparison with *P. aeroginosa* and *S. typhi*. Fig. 5. demonstrates the similar extended results for different concentrations of CdSe nanoparticles antibacterial activity and it can be seen that the same results obtained.

Table 2: Zone of inhibition

CdSe:Ag concentration (mg/ml)	*S. aureus* (mm)	*P. aeroginosa* (mm)	*S. typhi* (mm)
13.4	29	27	23
8.4	27	24	20
6.7	27	23	19
4.2	25	21	18
3.35	25	20	17
2.1	24	18	16
1.675	23	17	13
1.05	20	15	13

CONCLUSIONS

In this study, CdSe:Ag QDs in different concentrations were synthesized by chemical methods (2-3 nm). The size, structure and optical properties were assessed; and the antibacterial effects of them on the three bacteria (*S. aureus, P. aeroginosa* and *S. typhi*) were studied using Well diffusion and Macro dilution. The antibacterial activity of CdSe:Ag nanoparticles was assessed by the disc and well diffusion agar methods and the results show the antibacterial effect of CdSe:Ag nanoparticles. In fact, by increasing the nanoparticle concentration in wells and discs, the growth inhibition and diameter of inhibition zone has also been increased. The size of inhibition zone was different according to the type of bacteria and the concentrations of CdSe:Ag QDs and the maximum diameter has happened for *S. aureus.*

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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