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RESEARCH PAPER

Synthesis and characterization of CdO/GrO nanolayer for in vivo imaging

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ABSTRACT

Objective(s): Nanomaterials are playing major roles in imaging by delivering large imaging payloads, yielding improved sensitivity. Nanoparticles have enabled significant advances in pre-clinical cancer research as drug delivery vectors. Inorganic nanoparticles such as CdO/GrO nanoparticles have novel optical properties that can be used to optimize the signal-to-background ratio. This paper reports on a novel processing route for preparation of CdO/GrO nanolayer and investigation of its optical properties for application in in vivo targeting and imaging.

Materials and Methods: Nanostructures were synthesized by reacting cadmium acetate and graphene powder. The effects ofdifferent parameters such as power and time of irradiation were also studied. Finally, the efficiency of CdO/GrO nanostructures as an optical composite was investigated using photoluminescence spectrum irradiation. CdO/GrO nanostructures were characterized by means of X-ray diffraction (XRD), atomic force microscopy (AFM), scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) and photoluminescence (PL) spectroscopy.

Results: According to SEM images, it was found that sublimation temperature had significant effect on morphology and layers. The spectrum shows an emission peak at 523 nm, indicating that CdO/GrO nanolayer can be used for in vivo imaging.

Conclusion: The estimated optical band gap energy is an accepted value for application in in vivo imaging using a QD–CdO/GrO nanolayer.

Keywords: *CdO/GrO, Hexagonal nanostructures, In vivo targeting, Optical investigation*

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INTRODUCTION

The recent discovery of graphene has been accompanied with increasing research attention to explore this new material for drug delivery applications.

Due to its unique structure and geometry, graphene possesses remarkable physical-chemical properties including high Young's modulus, high fracture strength, excellent electrical and thermal conductivity, fast mobility of charge carriers, large specific surface area and biocompatibility [1-5].

The development of fluorescence and bioluminescence imaging approaches started in the 1990s with fluorescence reflectance imaging. These properties enable graphene to be considered as an ideal material for broad applications, ranging from quantum physics, nanoelectronics, energy research, catalysis and engineering of nanocomposites and biomaterials [6-9]. In the area of nanomedicine, graphene and its composites have emerged as new biomaterials which provide exciting opportunities for the development of broad applications including a new generation of biosensors, nano-carriers for drug delivery, and probes for cell and biological imaging [10-14]. Quantum dots (QDs), tiny light-emitting particles on the nanometer scale, are

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emerging as a new class of fluorescent probe for in vivo biomolecular and cellular imaging. In comparison with organic dyes and fluorescent proteins, QDs have unique optical and electronic properties including size-tunable light emission, improved signal brightness, resistance against photobleaching, and simultaneous excitation of multiple fluorescence colors. Recent advances have led to the development of multifunctional nanoparticle probes that are very bright and stable under complex in vivo conditions. A new structural design involves encapsulating luminescent QDs with amphiphilic block copolymers and linking the polymer coating to tumor-targeting ligands and drug delivery functionalities. Polymer-encapsulated QDs are essentially nontoxic to cells and animals, but their long-term in vivo toxicity and degradation need more careful study. Bioconjugated QDs have raised new possibilities for ultrasensitive and multiplexed imaging of molecular targets in living cells, animal models and possibly in humans [15].

Materials and physical measurements

All the chemical reagents used in our experiments were of analytical grade and were used as received without further purification. Graphite powders were used from Alfa (natural, briquetting grade, 10 meshes, 99.9995%) [16]. Graphene oxide powders were prepared following Staudenmaier's method and reduced to graphene powders by annealing at 1050 ◦C under an argon atmosphere [17]. XRD patterns were recorded by a Rigaku D-max C III, X-ray diffractometer using Ni-filtered Cu Kα radiation of Kashan-Iran University. Microscopic morphology of products was visualized by SEM (LEO 1455VP). Transmission electron microscopy (TEM) images were obtained on a Philips EM208 transmission electron microscope with an accelerating voltage of 200 kV. UV-Vis diffuse reflectance spectroscopy analysis was carried out using Shimadzu UV-2600 UV–Vis spectrophotometer with an integrating sphere attachment and $BaSO_4$ was used as reference.

Synthesis of Cd (OAc)$_2$ Nanostructures

In this work, cadmium (II) acetate powder was used as the starting reagent. $Cd(OAc)_2$ nanostructure as precursor was prepared in a vertical quartz pipe set in vacuum condition. Each experiment was carried out by loading 1 g of cadmium (II) acetate powder, which was transferred to the external pipe of the set. Then, the system was vacuumed by a pump. Afterwards, water entered the inner pipe from one side and exited from the other side. Water was circulated in the system in order to solidify the product vapors. The resulting product was gradually heated to the desirable temperature 130 °C for 120 min. After the heating process, the precipitations at the external part of the inner pipe were collected.

Synthesis of CdO/GrO nanolayer

In a typical experiment, 1 g of the as-obtained $Cd(OAc)_2$ nanostructure and 0.08 g graphene were loaded into a silicon boat that was later put in a high-temperature tube furnace. The sample was heated in air at 330 °C for 120 min. After the thermal treatment, the system was allowed to cool to room temperature and the obtained precipitations were collected.

In vivo study

For observing the overall effects of optical properties CdO/GrO nanolayer on a living subject, 15 ppm, 30 ppm, and 45ppm of QDs was injected to rat-tail as cell 'taggants' for in vivo imaging. After that, we put rat-tail exposed to UV irradiation for investigation of In vivo study.

RESULTS AND DISCAUSION

Physicochemical properties of CdO/GrO nanolayer

The XRD results indicated that pure cubic CdO/GrO nanoparticles without any impurities could be obtained after thermal decomposition at 330 °C for 2 h. All reflection peaks of the XRD pattern for CdO/GrO nanoparticles are indexed

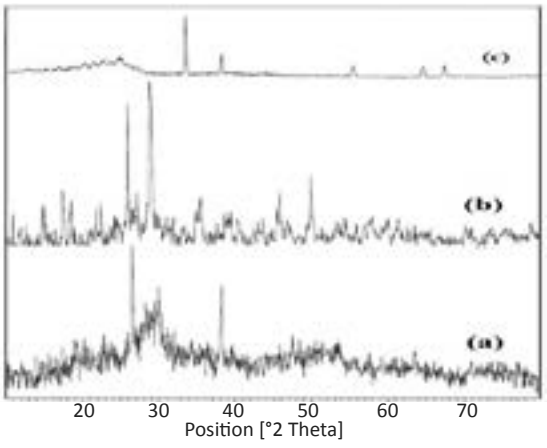

Fig. 1. XRD pattern of a) Cd(OAc)2 nanostructure and b) CdO/GrO nanolayer sublimated at 330 °C for 120 min and c) XRD pattern of graphene

well to orthorhombic phase with calculated cell parameters a = 3.3110˚A and b = 5.5260A˚.

XRD analysis is based on constructive interference of monochromatic X-rays and a crystalline sample. The characteristic x-ray diffraction pattern generated in a typical XRD analysis provides a unique "fingerprint" of the crystals present in the sample. XRD pattern of a) Cd $(OAc)_2$ nanostructure and b) CdO/GrO nanolayer sublimated at 330 °C for 120 min and c) XRD pattern of graphene are shown in Fig. 1.

SEM Images of samples show nanolayer structures have been produced. Transmission electron microscopy (TEM) image of CdO/GrO nanolayer structures are well correlated with the scanning electron microscopy image. Dynamic light scattering (DLS) diagram of CdO/GrO nanolayers shows size range between 95 to 100 nm. Photoluminescence (PL) spectroscopy shows the emission peak at 523 nm.

As shown in Fig. 2a, highly aggregated particles have been obtained by sublimation at 130 °C and in many parts micro and agglomerated nanostructures have been formed. By increasing temperature to 330 °C for the synthesis of CdO/GrO nanostructures (Fig 2b), morphology changed and the nanolayer structures were produced. According to Figs 2a-b, it was found that sublimation temperature has significant effect on layers morphology.

Calcinating the samples with different morphologies, produced structure with different sizes and the finest particles. These results demonstrate that the morphology of the starting reagent has remarkable effect on product size (i.e. starting reagent with specific morphology leads to a product with specific morphology).

To further investigate the details of the morphology, TEM images were taken. To prepare the TEM sample, the powder was dispersed in highly pure ethanol via ultrasonication for 20 min. TEM image of CdO/GrO nanoparticles synthesized from the $Cd(OAc)_2$ sublimated at 130 °C are shown in Fig. 3. The AFM image of CdO/

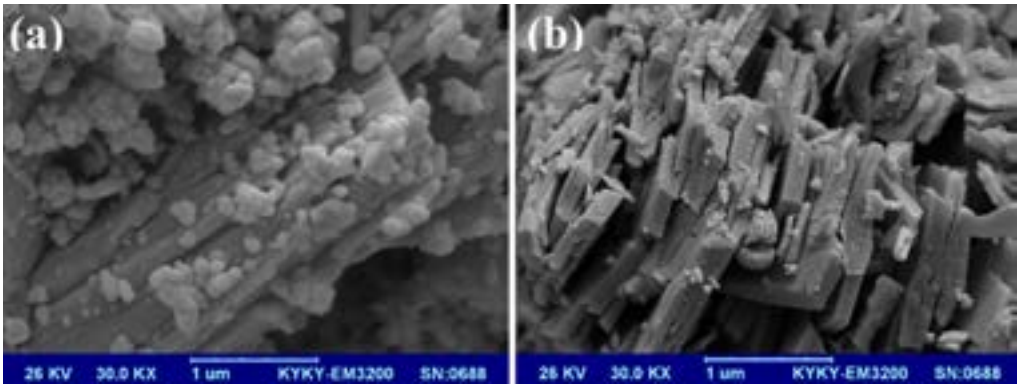

Fig. 2. SEM images of the Cd(OAc)2 sublimated at 130 °C (a) and CdO/GrO nanolayers sublimated at 330 °C

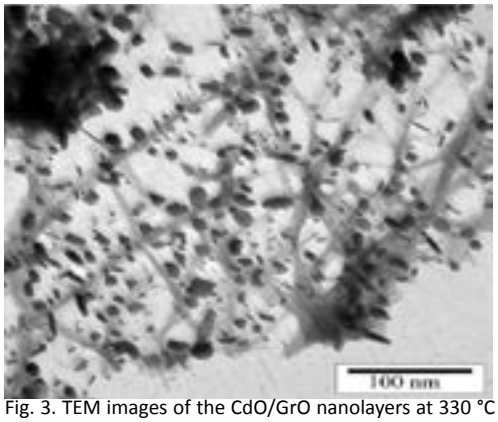

Fig. 3. TEM images of the CdO/GrO nanolayers at 330 °C for 120 min

GrO nanostructures are presented in Fig. 4 which clearly reveals the nanoparticles aggregates. Dynamic light scattering (DLS) was used to study the hydrodynamic size of nanolayers. DLS data of CdO/GrO nanostructures are shown in Fig. 5.

Optical property of the CdO/GrO nanoparticles under difference reaction conditions was investigated by photoluminescence (PL) spectroscopy and the results are given in Fig. 6. The spectrum shows the emission peak at 523 nm. Importantly, the PL properties were the same irrespective of the different sizes of the CdO/GrO nanostructures. Such a large blue shift of excitonic absorption band can be attributed to the small crystallite size of the samples [19].

In vivo imaging using CdO/GrO nanolayers in mice

For in vivo imaging study, we put rat-tail exposed to UV irradiation between 200nm-800nm.

fig. 7 shows the UV–Vis spectrum of CdO/GrO nanostructures and graphite structures, reflecting variation of % absorbance of CdO/GrO nanostructures as a function of wavelength. Broad peak around 398 nm indicates that the particle possesses quantum confinement.

One method for in vivo optical imaging employes a two-dimensional planar approach, akin to in vitro epifluorescence microscopy, which allows researchers to explore surface tissues and bones of an animal with fluorescence or bioluminescence.

By using the in vivo imaging with CdO/GrO nanostructures, we investigated light emission of CdO/GrO nanostructures from the circulation within minutes after injection. The schematic of methods was displayed in Fig 8.

It was found that CdO/GrO nanostructures had the ability to track and target cells by photoluminsence properties. QDs were found to

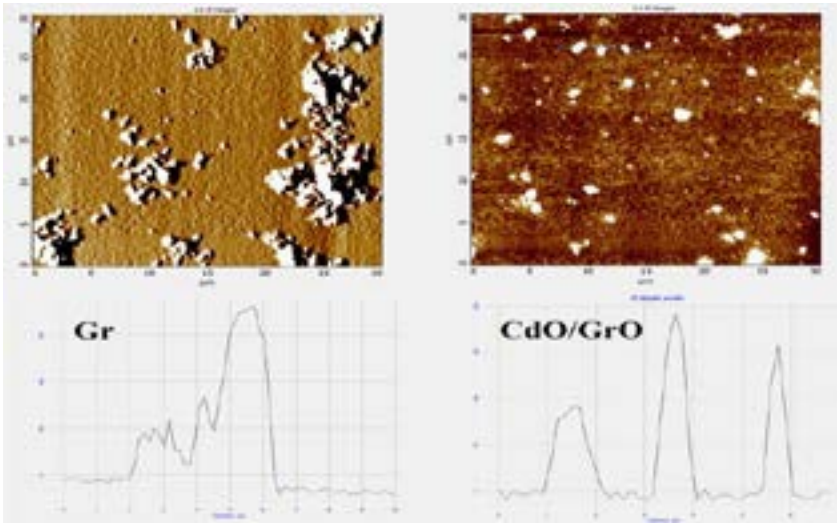

Fig. 4. AFM images of Grapphene and the CdO/GrO nanolayers

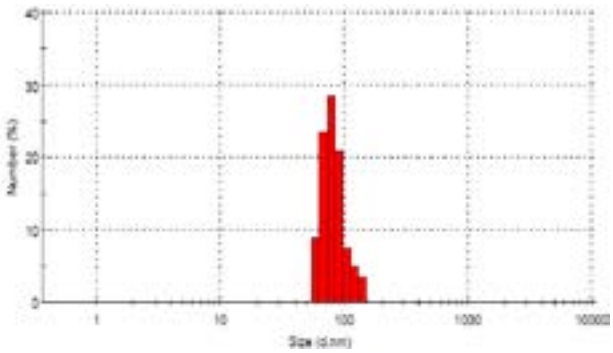

Fig. 5. Size distribution obtained by DLS analysis of CdO/GrO nanolayers

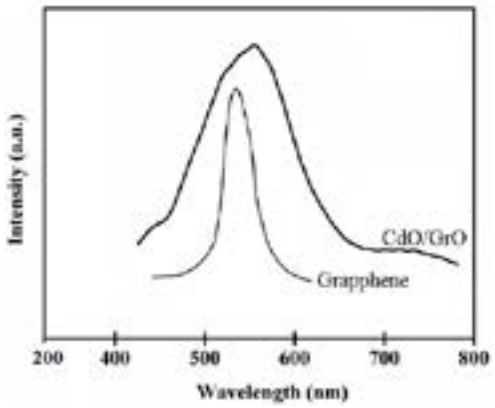

Fig. 6. Room temperature PL spectra of Graphene and CdO/GrO nanolayers

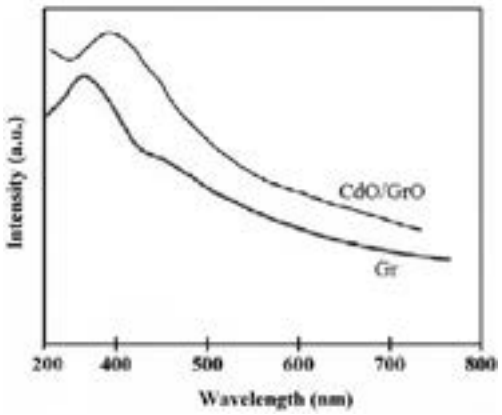

Fig. 7. UV–Vis spectra of Graphene and CdO/GrO nanolayers

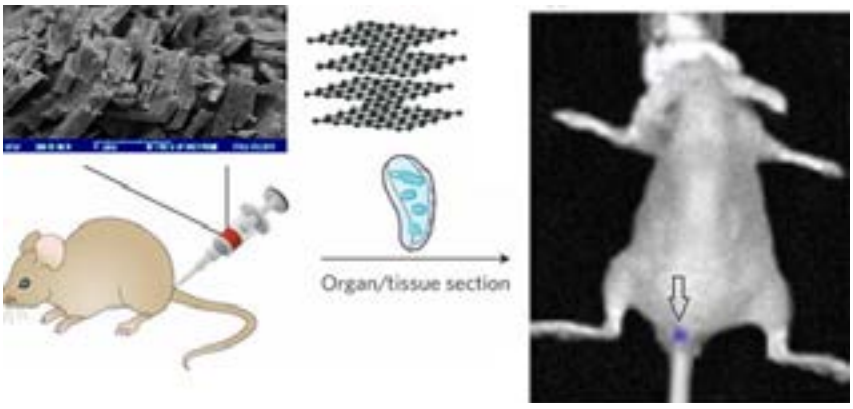

Fig. 8. In vivo imaging using CdO/GrO nanolayers in mice. Injection of QDs to rat-tail and radiation Uv for The estimated optical band gap energy

stay in the blood circulation and for in vivo optical imaging for an extended period of time (half-life more than 3 h). This long-circulating can be explained by the exclusive structural properties of QD nanoparticles. In vivo studies also confirmed the nontoxic nature of stably protected QDs [18].

Indeed, in vivo toxicity is haply to be a key factor in characterize whether QD imaging probes would be approved by regulatory agencies for human clinical use.

The optical property of the produced nanoparticles was studied by measuring the % absorbance and band gap energy.

The estimated optical band gap energy is an accepted value for application in in vivo targeting and imaging.

Gao [20] reported a new class of multifunctional QD probes for simultaneous targeting and imaging of tumors in live animals. Under in vivo conditions, QD probes can be delivered to tumors either by a passive targeting mechanism or through an active targeting mechanism.

In the passive mode, macromolecules and nanometer-sized particles are accumulated preferentially at tumor sites through an enhanced permeability and retention effect [21-27].

CONCLUSION

In this manuscript, CdO/GrO nanostructures have been successfully synthesized through a solvent-less solid-state thermal decomposition of cadmium (II) acetate nanostructures obtained by the sublimation of cadmium (II) acetate powder. We investigated the influence of the sublimation temperature on the morphology of $Cd(OAc)_2$ nanoparticles and consequently on the CdO/GrO nanoparticles.

The characteristics of products were deter-

mined by using XRD, SEM, PL, AFM and TEM analysis. The XRD results indicated that pure cubic CdO/GrO nanoparticles without any impurities obtained after thermal decomposition at 330 °C for 2 h.

Nanostructures injection to rats and their optical properties were investigation by UV–Vis spectroscopy. This study indicated that new composites developed in the current study are suitable materials for improved biocompatibility and circulation half-life.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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