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Fabrication of protein-coated CdS nanocrystals via microwave-assisted hydrothermal method

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CdS nanocrystals coated with protein were synthesised in the aqueous solution of bovine serum albumin protein by a rapid microwave-assisted hydrothermal method. Transmission electron microscopy, X-ray powder diffraction, Fourier transform infrared spectroscopy, thermogravimetric analysis (TGA) and cell cytotoxicity were used to characterise the morphology, composition and properties of the nanocrystals. The results indicate that the as-prepared CdS nanocrystals were wrapped up with the protein. The cytotoxicity profile on the human cervical cancer cell line HeLa shows that the potential cellular toxicity of the nanocrystals is efficiently prevented due to the presence of protein coating, which may offer the possibility of applications in biomedical research.

Keywords: cadmium sulphide; nanocrystal; biocompatible; hydrothermal

1. Introduction

Chalcogenide semiconductors have attracted broad attention in the past few decades due to their photoluminescent properties and other useful physical and chemical properties [1–3]. Among them, cadmium sulphide (CdS) is an excellent material with a direct band gap of 2.42 eV, which is now widely used for optoelectronic conversion devices, biolabelling and other bio-application [4–6]. However, cadmium-based nanocrystals are more likely to decompose partially and release highly toxic Cd^{2+} ions, which are fatal to cells [7–9]. A direct way to avoid the toxicity of Cd-based nanocrystals is to make them well coated to become biologically inert [10]. Many shells such as ZnS, silica, DNA and apoferritin have been proposed for the coating of Cd-based nanocrystals [9,11,12]. However, the procedures are generally complicated. It is still a challenge for chemists and material scientists to find more convenient routes for Cd-based nanocrystals with good biocompatibility.

In this study, protein-coated CdS nanocrystals were synthesised in the bovine serum albumin (BSA) aqueous solution by a rapid microwave-assisted hydrothermal method, which is proved to be very advantageous for the preparation of uniform nanocrystals [13,14]. To the best of our knowledge, this is the first report on microwave-assisted hydrothermal synthesis of protein-coated CdS nanocrystals. The results indicate that the nanocrystals exhibit good biocompatibility due to the presence of BSA coating.

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2. Experimental section

2.1. *Materials*

BSA (purity $\geq 98\%$) was purchased from Sigma-Aldrich. Thioacetamide (TAA, $\geq 99.0\%$) and other chemical reagents were of analytical grade and used as received without further purification.

2.2. *Fabrication of the CdS nanocrystals*

A microwave synthesis system (CEM Discover) made by CEM Instruments (USA) was used in this research. In a typical synthesis, 10 mL of 50 mM cadmium nitrate aqueous solution and 20 mL of 1 mg/mL BSA aqueous solution were mixed for 6 h. Then, 10 mL of 50 mmol/L TAA aqueous solution was added, and the mixed aqueous solution was treated at 200°C for 30 min. The microwave-accelerated reaction system was operated at a power of 200 W. The yellow precipitates were washed several times and dried for characterisation and application.

2.3. *Cytotoxicity assay*

Growth inhibitory effect of CdS nanocrystals on HeLa cells was tested by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [9]. Briefly, HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with foetal bovine serum (10%, v/v), streptomycin (0.1 mg/mL) and penicillin (100 U/mL) in a humidified atmosphere with 5% CO_2 at 37°C. The cells were seeded in 96-well plates at 5×10^3 cells per well in DMEM and incubated overnight. The cells were then treated in triplicate with fresh medium containing grade concentrations (0.01, 0.05, 0.20 and 1.00 mg/mL) of the nanocrystals and incubated at 37°C for 6, 12, and 24 h. Aliquots of MTT solution (10 μL and 5 mg/mL) were added to each of the wells for 4 h of incubation. After the medium was removed, 150 μL of DMSO was added to each well. The absorbance of the purple formazan was recorded at 490 nm using an ELISA plate reader. The cytotoxicity results were calculated based on the data of three replicate tests.

2.4. *Characterisation*

X-ray powder diffraction (XRD) measurements were performed on a Japan Shimadzu XRD-6000 diffractometer with Cu Kα radiation ($\lambda = 0.15418$ nm). A scanning rate of $0.05^\circ\ s^{-1}$ was applied to record the patterns in the 2θ range of 10–80°. Transmission electron microscopy (TEM) images were taken using a JEOL JEM-2100 transmission electron microscope at an accelerating voltage of 200 kV, equipped with selected area electron diffraction (SAED) and X-ray energy dispersive spectroscopy (EDS). The Fourier transform infrared (FTIR) spectra were recorded on a Nicolet 6700 FTIR spectrograph in the wavenumber range of 4000–400 cm^{-1}. Thermogravimetric analysis (TGA) was performed on a Pyris 1 DTA instrument with a heating rate of 10°C/min in a nitrogen flow (20 mL/min).

3. Results and discussion

The crystal structure of the as-prepared nanocrystals was studied by XRD analyses. As shown in Figure 1, the sharp and strong peaks indicate that the sample exhibits good crystal quality. The diffraction pattern is consistent with that of hexagonal CdS phase (JCPDS Card File No. 41-1049). Calculations using the Debye–Scherrer formula for the strongest peak give a grain size of 25 nm.

The morphology of CdS were examined by TEM. As shown in Figure 2a, the nanocrystals display a uniform grain size of about 20 nm. The high-resolution TEM image in Figure 2b shows

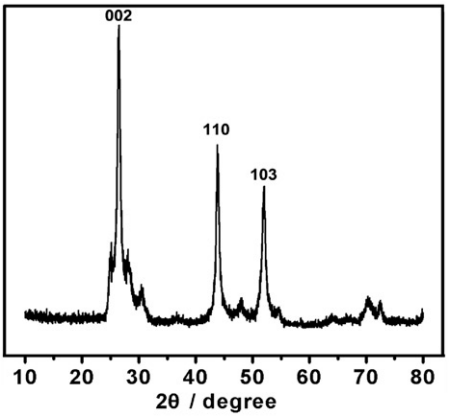

Figure 1. The XRD patterns of the as-prepared CdS nanocrystals.

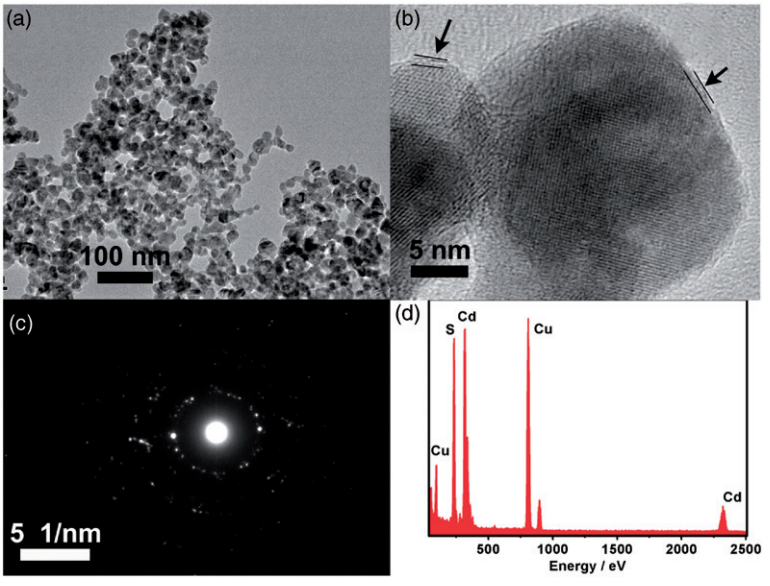

Figure 2. (a) Low-resolution TEM, (b) high-resolution TEM, (c) SAED pattern and (d) EDS spectra of the as-prepared CdS nanocrystals.

that the nanocrystals exhibit clear lattice fringes. More interestingly, a fuzzy and amorphous coat with an average thickness of about 1 nm (indicated by arrows) is visualised around each nanocrystal, suggesting that the nanocrystals may be coated with protein. The SAED pattern in Figure 2c reveals that the nanocrystals exhibit good crystallinity. The corresponding X-ray energy dispersive (EDS) spectroscopy in Figure 2d shows the significant presence of Cd and S with atomic ratio 1:1 approximately.

The surface composition of the CdS nanocrystals was determined by FTIR spectroscopy. As shown in Figure 3, the IR peaks of pure BSA at 3398, 1641 and 1528 cm^{-1} are assigned to the stretching vibration of –OH, amide I and amide II bands, respectively. Comparing with the IR

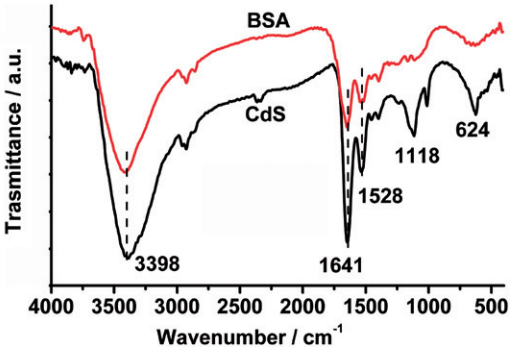

Figure 3. The FTIR spectra of pure BSA and CdS nanocrystals.

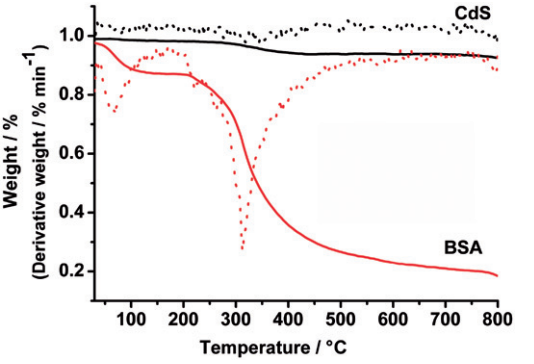

Figure 4. The TGA curves (—) and the corresponding DTG plots (···) of pure BSA and CdS nanocrystals.

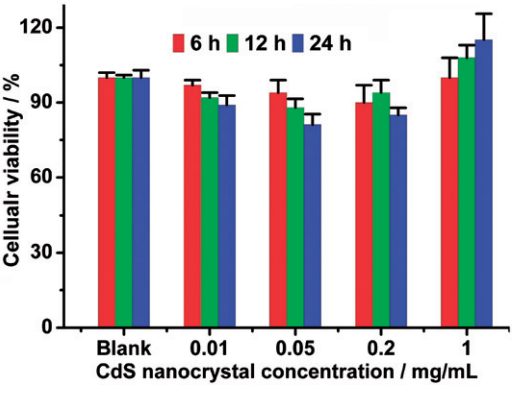

Figure 5. Cytotoxicity of the CdS nanocrystals with different concentrations of 0.01–1.00 mg/mL after 6, 12 and 24 h with untreated cells as a negative reference.

spectrum of BSA, the nanocrystals show the similar typical absorptions at the above three positions. These evidences confirm the existence of protein on the surface of CdS nanocrystals.

The involvement of the BSA protein in the CdS nanocrystals was investigated by TGA. As shown in Figure 4, in the testing temperature range (25–800°C), BSA loses ca 81.5% weight, whereas CdS nanocrystals only lose ca 7.5 % weight. DTG is useful for observing slight changes in weight, which are not readily noticed on the TGA thermograms. In DTG, the rate of per cent weight loss versus time (dw/dt) is obtained from the first derivative of the TGA curve [15]. As the dashed lines in Figure 4 show, BSA presents a very steep curve around 300°C, whereas CdS displays a relatively flat peak at the same position. The consistency of the inflection point at 300°C on the DTG plot of BSA and CdS nanocrystals suggests that the nanocrystals are a composite of BSA and CdS and contain the protein component with weight per cent accounting for ca 6%.

The cytotoxicity of the CdS nanocrystals was tested on the human cervical cancer cell line HeLa by MTT assay. As shown in Figure 5, the viability of untreated cells was assumed to be 100%. Over 80% cell viability was obtained when treated with 0.01–0.2 mg/mL of the nanocrystals. When the concentration was increased to 1.0 mg/mL, over 100% of the cellular

viabilty was observed. These results indicate that the nanocrystals exhibit lower cytotoxicity and even contribute to cell proliferation with increasing concentration. The proliferation effect of the nanocrystals may stem from the involvement of BSA because the protein BSA is a nutriment for many cells [16]. These evidences suggest that the potential toxic effect of CdS can almost be completely shielded by the protein coat, and hence, their biocompatibility is greatly enhanced.

4. Conclusions

In summary, CdS nanocrystals coated with the protein BSA were conveniently fabricated by the microwave-assisted hydrothermal method. BSA acts as a stabiliser for CdS mineralisation firstly and serves as a coating material for the formed nanocrystals subsequently. *In vitro* assay shows that the potential cellular toxicity of the nanocrystals is efficiently prevented by this approach. This work provides a new insight into the preparation of protein-coated nanomaterials, which would have great potential for biomedical applications such as bioimaging, targeted drug delivery, cancer diagnosis and microchip technology.

Acknowledgements

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References

[1] M. Amelia, R. Flamini, and L. Latterini, *Recovery of CdS nanocrystal defects through conjugation with proteins*, Langmuir 26 (2010), pp. 10129–10134.
[2] X.S. Niu, S.H. Liu, R.M. Xing, X.J. Wang, B.F. Zhang, J.J. Chen, and L. Yang, *Synthesis of single-crystalline silver sulfide nanowires by diffusion in porous anodic aluminium oxide template*, Mater. Lett. 61 (2007), pp. 5098–5101.
[3] A. Phuruangrat, T. Thongtem, and S. Thongtem, *Characterisation of one-dimensional CdS nanorods synthesised by solvothermal method*, J. Exp. Nanosci. 4 (2009), pp. 7–54.
[4] S.L. Xiong, B.J. Xi, and Y.T. Qian, *CdS hierarchical nanostructures with tunable morphologies: Preparation and photocatalytic properties*, J. Phys. Chem. C 114 (2010), pp. 14029–14035.
[5] L.A. Li, P.C. Wu, X.S. Fang, T.Y. Zhai, L. Dai, M.Y. Liao, Y. Koide, H.Q. Wang, Y. Bando, and D. Golberg, *Single-crystalline CdS nanobelts for excellent field-emitters and ultrahigh quantum-efficiency photodetectors*, Adv. Mater. 22 (2010), pp. 3161–3165.
[6] L.B. Hai, N.X. Nghia, P.T. Nga, V.D. Chinh, N.T.T. Trang, and V.T.H. Hanh, *Preparation and spectroscopic investigation of colloidal CdSe/CdS/ZnS core/multishell nanostructure*, J. Exp. Nanosci. 4 (2009), pp. 277–283.
[7] A.M. Derfus, W.C.W. Chan, and S.N. Bhatia, *Probing the cytotoxicity of semiconductor quantum dots*, Nano. Lett. 4 (2003), pp. 11–18.
[8] Y. Su, Y. He, H. Lu, L. Sai, Q. Li, W. Li, L. Wang, P. Shen, Q. Huang, and C. Fan, *The cytotoxicity of cadmium based, aqueous phase synthesized, quantum dots and its modulation by surface coating*, Biomaterials 30 (2009), pp. 19–25.
[9] R.M. Xing, X.Y. Wang, L.L. Yan, C.L. Zhang, Z. Yang, X.H. Wang, and Z.J. Guo, *Fabrication of water soluble and biocompatible CdSe nanoparticles in apoferritin with the aid of EDTA*, Dalton Trans. (2009), pp. 1710–1713.
[10] Y. Su, M. Hu, C. Fan, Y. He, Q. Li, W. Li, L.-h. Wang, P. Shen, and Q. Huang, *The cytotoxicity of CdTe quantum dots and the relative contributions from released cadmium ions and nanoparticle properties*, Biomaterials 31 (2010), pp. 4829–4834.
[11] N. Ma, J. Yang, K.M. Stewart, and S.O. Kelley, *DNA-passivated CdS nanocrystals: Luminescence, bioimaging, and toxicity profiles*, Langmuir 23 (2007), pp. 12783–12787.
[12] H. Goesmann and C. Feldmann, *Nanoparticulate functional materials*, Angew. Chem. Int. Ed. 49 (2010), pp. 1362–1395.
[13] S.H. Liu, F. Lu, X.D. Jia, F.F. Cheng, L.P. Jiang, and J.J. Zhu, *Microwave-assisted synthesis of a biocompatible polyacid-conjugated Fe_3O_4 superparamagnetic hybrid*, Crystengcomm. 13 (2011), pp. 2425–2429.
[14] S.H. Liu, F. Lu, and J.J. Zhu, *Highly fluorescent Ag nanoclusters: Microwave-assisted green synthesis and Cr^{3+} sensing*, Chem. Commun. 47 (2011), pp. 2661–2663.

[15] R.M. Xing, X.Y. Wang, C.L. Zhang, J.Z. Wang, Y.M. Zhang, Y. Song, and Z.J. Guo, *Superparamagnetic magnetite nanocrystal clusters as potential magnetic carriers for the delivery of platinum anticancer drugs*, J. Mater. Chem. 21 (2011), pp. 11142–11149.
[16] R.M. Xing, S.H. Liu, and S.F. Tian, *Microwave-assisted hydrothermal synthesis of biocompatible silver sulfide nanoworms*, J. Nanopart. Res. 13 (2011), pp. 4847–4854.