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Facile and controlled synthesis of stable water-soluble cupric sulfide quantum dots for significantly inhibiting proliferation of cancer cells

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Amorphous and crystalline copper sulfide quantum dots (QDs) with good water-solubility were obtained controllably by a novel hydrolysis strategy. These QDs exhibit anti-proliferation activities on cancer cells rather than normal cells and the biological activities are related to their polymorphs. Our study opens new avenues for fabricating stable water-soluble metal sulfide QDs.

Metal sulfide quantum dots (MSQDs) semiconductor materials have been drawing considerable attention, due to their specific properties and potential applications[1, 2], such as in electronics[3], optics[4], catalysis[5], magnetic storage[6], and so on. Especially some MSQDs exhibit good photoluminescent properties[7] and cytotoxicities in cancer cells[8], enabling them to express potential applications in biomedical[9, 10] and pharmaceutical fields[11], and showing an expansive developing prospects. However, because most biological applications occur in the aqueous media, it is necessary to transfer the QDs into the aqueous media firstly during the biological applications. The previous reports have indicated that the water-soluble QDs rather than the oil-soluble QDs exhibit the good biocompatibilities[12, 13]. Thus it is the great importance to the synthesis of stable water-soluble QDs. However, up to now, the synthesis of stable water-soluble QDs is still confronted with many difficulties. The reasons are: (1) many water-soluble QDs are not stable and liable to aggregate without surface modification in the aqueous phase, (2) their crystallinities are poor, resulting in that their some performance and applications are affected, (3) the yield is low, leading to some difficulties in practical application.

As a kind of typical metal chalcogenide semiconductors, cupric sulfide (CuS) exhibits nearly ideal solar control characteristics[14] and good biological effects[15]. Therefore, the study of CuS QDs has aroused research interests in recent years. So far, there have been some methods to fabricate CuS QDs, such as electrochemical synthesis[16], hot-injection approach[17, 18], cation exchange reactions[19], hydrothermal method[20, 21, 22], *etc*. However, theses prepared water-insoluble QDs have to be modified to improve their water-solubility in order to be applied into the biological fields, which inevitably increase the complexity of the synthesis procedure. Therefore, the facile synthesis of stable water-soluble CuS QDs without any post-modification is still a great challenge.

In this communication, we demonstrate a facile strategy to synthesize the stable amorphous and crystalline water-soluble CuS QDs. This method exhibits remarkable advantages. Firstly, the hydrosols of the as-prepared CuS QDs are significantly stable without using any surface modification reagents. Secondly, the size and the crystal polymorphs can be controlled by adjusting the reaction time and temperature. Thirdly, the as-prepared CuS QDs show the anti-proliferation effects on human cancer cells rather than normal cells. Finally, this synthesis method is simple, low cost, easily scalable and can be expanded to prepare other metal chalcogenides QDs such as CdS, ZnS QDs (Fig. S1, ESI†).

In this paper, the amorphous and crystalline CuS QDs were prepared through a two-step method (ESI†). In a typical procedure, firstly, a milk turbid precursor was prepared through mixing the ethanol solutions of cupric chloride ($CuCl_2$) and thiaocetamide (TAA). During the process, the cupric ion (Cu^{2+}) was reduced into the cuprous ion (Cu^{+}) in the precursor. From the X-ray diffraction (XRD) analysis (Fig. S2, ESI†), the precursor is composed of $Cu_3(TAA)_3Cl_3$[23]. This reaction can be described as equation (1):

$$CuCl_2 + TAA + CH_3CH_2OH \rightarrow Cu_3(TAA)_3Cl_3 + H^+ + NH_4^+ + Cl^- + SO_4^{2-} + CH_3COO^- \quad (1)$$

Secondly, the products were prepared by the hydrolysis of the precursor. The precursor was added into distilled water and CuS QDs with different crystal polymorphs were synthesized by controlling the reaction time and temperature. The amorphous and crystalline CuS QDs were fabricated at 5 ℃ for 24 h and at 40 ℃ for 30 min, respectively. During the preparation process the light yellow turbidity dispersion gradually turned into the yellowish-brown transparent hydrosol for amorphous product and into black green transparent hydrosol for crystalline product. This reaction can be described as equation (2):

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$$Cu_3(TAA)_3Cl_3 + O_2 + H_2O \rightarrow CuS + NH_4^+ + H^+ + Cl^- + CH_3COO^- \quad (2)$$

XRD patterns were recorded and were used to demonstrate the formation of the amorphous and crystalline CuS QDs (Fig. 1). From Fig. 1a, the absence of any obvious diffraction peaks indicates that the samples fabricated at 5 ℃ for 24 h are amorphous. From Fig. 1b, the samples exhibit diffraction peaks with the 2θ values of 29.454°, 31.819°, 48.103°, which could be indexed to the (102), (103) and (110) planes of the hexagonal covellite CuS (JCPDS No. 01-1281, a = 3.802 Å and c = 16.43 Å). This reveals that the samples fabricated at 40 ℃ for 30 min are crystals. The average grain size of the nanocrystals calculated along the (110) plane using the Scherrer formula is about 5.2 nm.

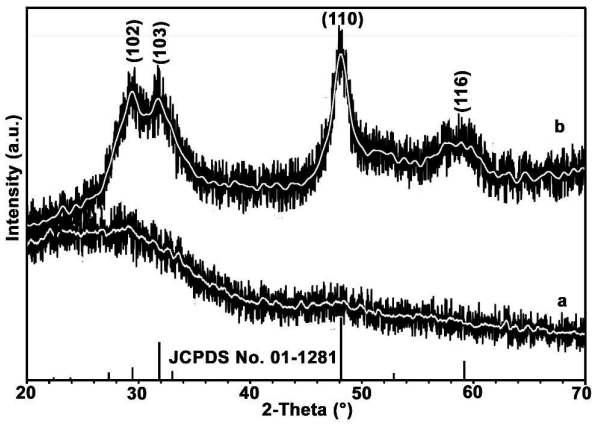

Fig. 1 XRD patterns of CuS QDs. (a) amorphous, (b) crystals.

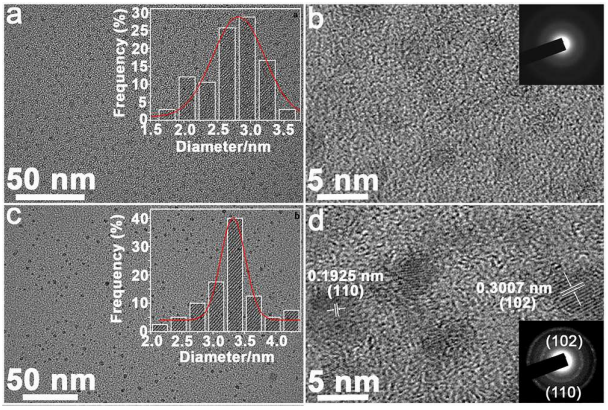

Fig. 2 HRTEM , SAED (inset) and particle sizes distribution (inset) of CuS QDs, (a) and (b) amorphous, (c) and (d) crystals.

Fig. 2 shows the high resolution transmission electron microscope (HRTEM) images of the amorphous and crystalline CuS QDs. Particle size distributions and selected area electron diffractions (SAED) images are shown in the insets. From Fig. 2a and Fig. 2c, the as-prepared QDs are well-dispersed. Furthermore, both of the QDs exhibit the relatively narrow particle size distribution with the average diameter of 2.7 nm for amorphous QDs and 3.3 nm for crystalline QDs. From Fig. 2b, the existence of diffused halo ring rather than any detectable rings or spots in the SAED image further reveals the formation of the amorphous product, which agrees well with the XRD result (Fig. 1a). From Fig. 2d, the HRTEM shows the lattice fringe spacing are 0.30 nm and 0.19 nm. Based on the SAED (inset of Fig. 2d), the lattice fringe spacing of the inside and outside ring are also calculated as 0.30 nm and 0.19 nm, corresponding to the (102) and (110) planes of the hexagonal covellite CuS. These results further confirm the formation of the amorphous and crystalline CuS QDs.

In the current study, Zeta potential analysis results demonstrate the stability of the QDs hydrosols. From the results shown in Fig. 3, the zeta potentials of amorphous and crystalline CuS QDs hydrosols are both about -30 mV, indicating the relative stability of CuS QDs in neutral deionized water [24]. Additionally, very clear and stable hydrosols of CuS QDs can be obtained when dispersed into neutral deionized water. Furthermore, the zeta potentials of the CuS QDs in different physiological solutions including Phosphate Buffer solution (PBS), Fetal Bovine Serum(FBS), and Dulbecco Modified Eagle Medium (DMEM) are also determined. From the results, the zeta potentials of amorphous and crystalline CuS QDs are -27.0 mV and -23.2 mV in PBS, -21.3 mV and -19.6 mV in FBS, -24.7 mV, and -21.8 mV in DMEM, respectively (Table S1, ESI†). These result shows the zeta potentials of amorphous QDs are more negative than those of the crystalline QDs, indicating the better stability of amorphous CuS QDs in these physiological solutions.

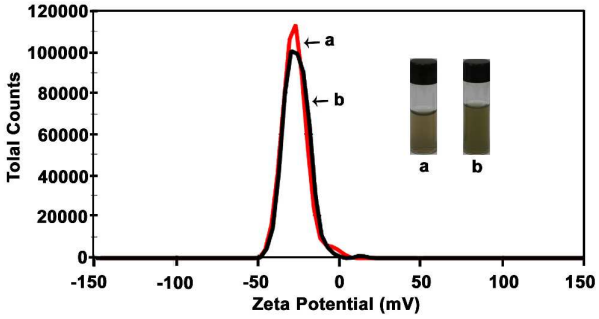

Fig. 3 The zeta potentials of CuS QDs. The red line is amorphous (a), the black line is crystals (b).

The solid photoluminescence (PL) determination indicates the good PL properties of the CuS QDs. From the solid PL spectra of CuS QDs shown in Fig. 4, the QDs exhibit the emission between 375 and 600 nm when excited with light at a wavelength of 330 nm. The amorphous CuS QDs have a maximum intensity at approximately 459 nm (right of Fig. 4a), while the crystalline CuS QDs have a maximum intensity at approximately 461 nm (right of Fig. 4b), agree well with the literature[25]. Moreover, the PL analysis result indicates that the PL intensity of the crystalline CuS QDs is higher than that of the amorphous CuS QDs. In order to test the PL properties of CuS QDs further, the excitation spectra were measured. The emission wavelength is set in 460 nm. From the results (left of Fig. 4), the maximum absorptions are at 331 nm for amorphous QDs and 335 nm for crystalline QDs. This result agrees well with the above-mentioned emission result, further confirming the PL properties of the CuS QDs. In order to investigate the PL stability, the PL spectra of the CuS QDs were measured at different time (Fig.S4, ESI†).The result shows that the intensity of QDs rarely decreased even after 300 min, indicating the relatively good PL stability of the CuS QDs.

The fluorescence decay of the CuS QDs was measured using time-correlated single photon counting (TCSPC) under the excitation of a

pico-second (ps) 405 nm laser pulse. The time-resolved luminescence decay plots in Fig. 5 could be fitted with a biexponential decay probabilities for the two levels. For amorphous

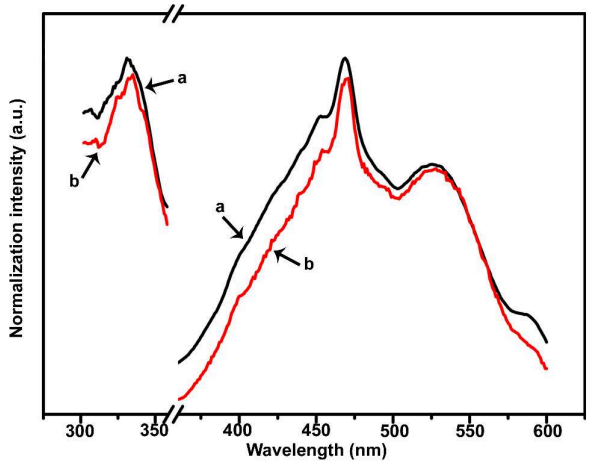

Fig. **4** PL spectra of CuS QDs, (a) amorphous, (b) crystals.

CuS QDs, the first decay has a lifetime of 0.52 ns and the second decay has a lifetime of 4.61 ns. For crystalline CuS QDs, the first decay has a lifetime of 0.47 ns and the second decay has a lifetime of 5.14 ns. The decays can be attributed to the initially populated core-state recombination. The results consistent with the theoretically calculated value of 3 ns when the screening of the radiating field inside the QD is taken into account.[26]

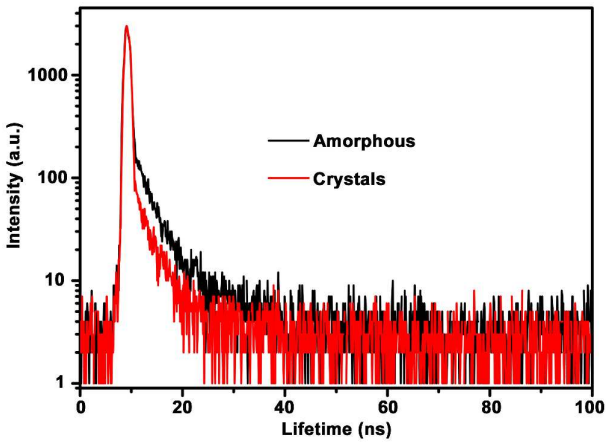

Fig. 5 Time-resolved photoluminescence of CuS QDs. (a) amorphous, (b) crystals.

The anti-proliferation activities of amorphous and crystalline CuS QDs were evaluated through MTT assay on human cervical cancer cells (Hela cells, Fig. 6a), human hepatocellular carcinoma cells (Hep G2 cells, Fig. 6b), glioma cells (s180 cells, Fig. 6c) and hamster normal cells (V79 cells, Fig. 6d), respectively. The IC_{50} values (half maximal inhibitory concentration) of amorphous and crystalline CuS QDs are 17.78 $\mu g \cdot mL^{-1}$ and 22.91 $\mu g \cdot mL^{-1}$ on Hela cells, 9.42 $\mu g \cdot mL^{-1}$ and 17.62 $\mu g \cdot mL^{-1}$ on Hep G2 cells, 1.62 $\mu g \cdot mL^{-1}$ and 7.59 $\mu g \cdot mL^{-1}$ on s180 cells, respectively. However, the IC_{50} values of amorphous and crystalline CuS QDs on V79 cells are not available, indicating that they just inhibit the proliferation of V79 cells slightly. These results reveal that the CuS QDs exhibit higher anti-proliferation activities on human cancer cells than normal cells, and the anti-proliferation activities of amorphous CuS QDs on cancer cells are stronger than those of the crystalline QDs. The amorphous QDs should be more active and comparatively soluble than crystalline QDs and might have much more active sites on the particle surface. This might be the reason for the stronger effects of amorphous CuS QDs.

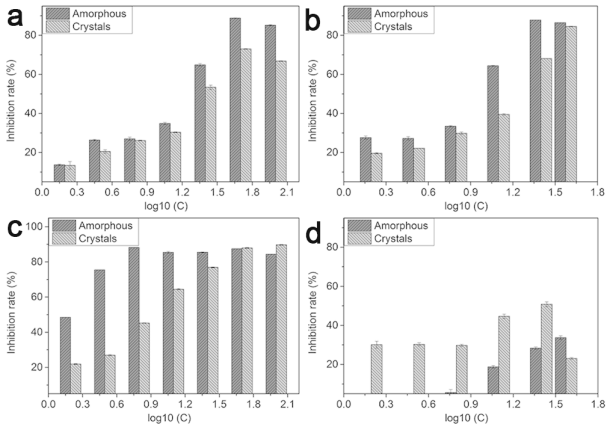

Fig. **6** Anti-proliferation effects of amorphous CuS QDs, crystalline CuS QDs, on (a) Hela cells, (b) Hep G2 cells, (c) s180 cells, (d) V79 cells.

In order to investigate the mechanism of the anti-proliferation activities of CuS QDs on cells, the uptakes of amorphous and crystalline CuS QDs by Hep G2 cells and V79 cells were determined. The luminescent imaging was performed using confocal laser scanning microscopy (CLSM) and the intracellular luminescence was observed (Fig. 7). The bright-field images (a_1, b_1, c_1, d_1) and overlay of confocal luminescence (a_3, b_3, c_3, d_3) show that the luminescence can be observed in the cytoplasm but not in the membrane surface and nucleus. Compared the uptake of amorphous (a, c) with crystals (b, d) by cells, the uptake of amorphous are more than that crystalline CuS QDs. The more uptake of the amorphous CuS QDs cells than that of crystalline CuS QDs may result in their higher anti-proliferation activities on cells.

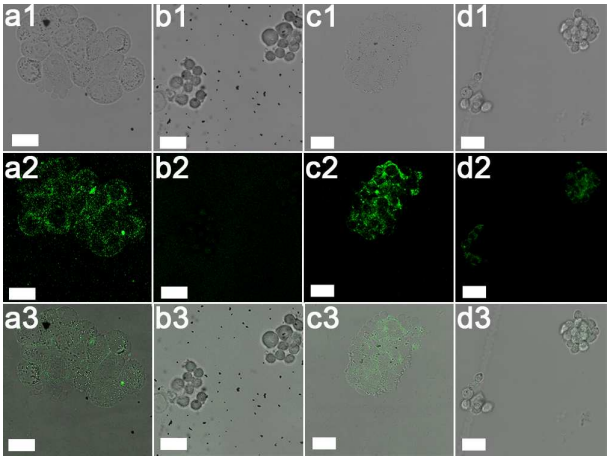

Fig. 7 CLSM images of cells treated with CuS QDs. (a) amorphous-Hep G2, (b) crystals-Hep G2, (c) amorphous-V79, (d) crystals-V79. 1.Bright-field images, 2. Luminescent images, 3. Overlay. Scale bar: 30 μm.

Because of the significant anti-proliferation effects of amorphous and crystalline CuS QDs on cancer cells, the apoptosis inducing effects of the CuS QDs on Hep G2 cells were determined through flow cytometric quadrant analysis. From the result (Fig. 8), both amorphous and crystalline CuS QDs can induce the apoptosis and necrosis of cancer cells. The result reveals the as-prepared QDs can inhibit the proliferation of cancer cells through inducing the apoptosis and necrosis of cancer cells. The LL, LR, UR, UL represent the normal live cells, early apoptotic cells, late apoptotic cells and necrotic cells, respectively.

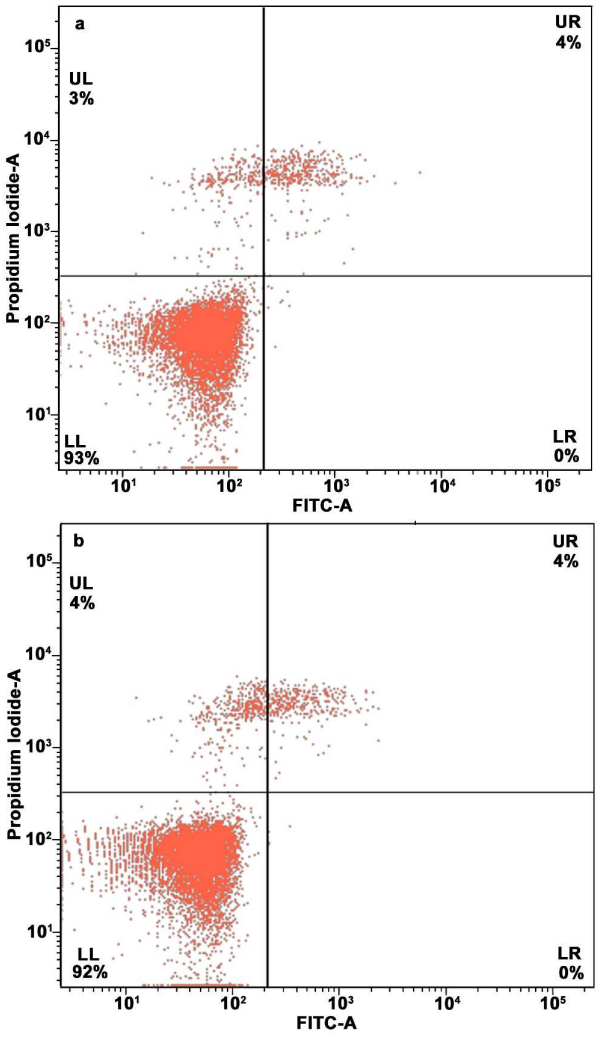

Fig. 8 Flow cytometric quadrant analysis of Hep G2 cells after treated with CuS QDs. (a) amorphous, (b) crystals.

Conclusions

In conclusion, the stable water-soluble CuS QDs have been prepared through a facile hydrolysis method. This method is a facile and manageable synthesis method for the water-soluble CuS QDs. Fluorescence test shows that the water-soluble CuS QDs exhibit good photoluminescent properties. Biological assays reveal that the QDs can enter into the cancer cells, and exhibit different anti-proliferation activities through apoptosis- and different anti-proliferation activities through apoptosis- and necrosis-inducing mechanisms. Their good water-soluble properties, optic properties and biological activities may make them used into biological and energy material field, such as they could be served as the new medical materials and the sensitizers in quantum dots sensitized solar cell. We also got other morphologies and sizes CuS by changing reaction conditions. What's more, we obtained other metal sulfide QDs in this way. Our synthesis opens new avenues for fabricating metal sulfide quantum dots.

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Notes and references

1 L. E. Brus, *Appl. Phys. A.*, 1991, **53**, 465–474.
2 Y. Wang, N. Heron, *J. Phys. Chem.*, 1991, **95**, 525–532.
3 C. J. Raj, B. C. Kim, *J. Alloy. Compd.*, 2014, **586**, 191–196.
4 H. S. Kim, K. B. Yoon, *Coordin Chem. Rev.*, 2014, 263–264.
5 C. M. Fan, M. D. Requlacio, C. Ye, S. H. Lim, Y. G. Zheng, Q. H. Xu, A.W. Xu, M. Y. Han., *Chem. Commun.*, 2014, **50**, 7128-7131.
6 F. L. Faita, K. Ersching, *J. Alloy. Compd.*, 2014, **590**, 176–183.
7 J. Y. Xin, P. Dong, *Colloids and Surfaces A: Physicochem. Eng. Aspects.*, 2014, **450**, 25–35.
8 L. Peng, M. He, B. B. Chen, *Biomaterials*, 2013, **34**, 9545-9558.
9 L. Carlini and J. L. Nadeau, *Chem. Commun.*, 2013, **49**, 1714-1716.
10 W. Kuzyniak, O. Adegoke, *Int. J. Pharmaceut.*, 2014, **466**, 382 – 389.
11 K. Yang, L. Zhu, L. M. Nie, X. L. Sun, L. Cheng, C. X. Wu, G. Niu, X. Y. Chen and Z. Liu, *Theranostics*, 2014, **4**, Issue 2.
12 S. M. M. Nelwamondo, M. J. Moloto, *Mater. Res. Bull.*, 2012, **47**, 4392–4397.
13 J. Liu, Z. Shi, Y. Yu, R. Yang, and S. Zue, *J. Colloid. Interf. Sci.*, 2010, **342**, 278.
14 R. S. Mane, C. D. Lokhande, *Mater. Chem. Phys.*, 2000, **65**, 1.
15 Y. M. Guo, J. Zhang, L. Yang, H. J. Wang, F. F. Wang and Z. Zheng., *Chem. Commun.*, 2010, **46**, 3493–3495.
16 Y. J. Yang, S. S. Hu, *J. Solid. State. Electr.*, 2008, **12**, 1405–1410.
17 A. E. Saunders, A. Ghezelbash, D.M. Smilgies, M. B. Sigman, B. A. Korgel, *Nano Lett.*, 2006, 6, 2959−2963.
18 X. Liu, X. Wang, B. Zhou, W. C. Law, A. N. Cartwright, M. T. Swihart, *Adv. Funct. Mater.*, 2013 , **23**, 1256−1264.
19 J. B. Rivest, P. K. Jain, *Chem. Soc. Rev.*, 2013, **42**, 89−96.
20 X. Y. Chen, Z. H. Wang, X. Wang, R. Zhang, X. Y. Liu,W. J. Lin and Y. T. Qian, *J. Cryst. Growth*, 2004, **263**, 570-574.
21 A. M. Qin, Y. P. Fang, H. D. Ou, H. Q. Liu and C. Y. Su, *Cryst. Growth Des.*, 2005, 5, 855–860.
22 P. Roy and S. K. Srivastava, *Cryst. Growth Des.*, 2006, **6** , 1921–1926.
23 Z. Y. Yao, X. Zhu, C.Z. Wu, X.J. Zhang, and Y. Xie, *Cryst. Growth Des.*, 2007, **7**, No. 7, 1256-1261.
24 V. B. Patravale, Abhijit A. Date and R. M. Kulkarni, *J. Pharm. Pharmacol.*, 2004, 56, 827–840.
25 Joyjit Kundu and Debabrata Pradhan, *ACS Appl. Mater. Inter.*, 2014, **6**, 1823−1834.
26. B. L. Wehrenberg, C. Wang, P. Guyot-Sionnest, *J. Phys. Chem. B* 2002, **106**, 10634.

Stable amorphous and crystalline copper sulfide quantum dots (QDs) were obtained controllably by a facile two-step method. These QDs exhibit anti-proliferation activities on cancer cells. This synthesis method is simple, low cost, easily scalable and can be expanded to prepare other metal chalcogenides QDs such as CdS, ZnS QDs.