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Size Controlled Synthesis of Germanium Nanocrystals by Hydride Reducing Agents and Their Biological Applications

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Germanium nanocrystals were synthesized by the hydride reduction of germanium tetrachloride ($GeCl_4$) in inverse micelles using hydride reducing agents including lithium aluminum hydride ($LiAlH_4$), lithium triethyl borohydride ($Li(C_2H_5)_3BH$), lithium borohydride ($LiBH_4$), and sodium borohydride ($NaBH_4$). Monodisperse Ge nanocrystals with average sizes ranging from 4.0 to 5.5 nm were produced using a simple room temperature technique. The nanocrystals were capped using allylamine. The nanocrystals displayed strong blue luminescence, and their application as optical probes is demonstrated by the imaging of HepG2 cells. Transmission electron microscopy (TEM), selected area electron diffraction (SAED), energy dispersive X-ray spectroscopy (EDX), and fluorescence spectroscopy were used to characterize the nanocrystals. The cytotoxicity of the nanocrystals is also reported, and the germanium quantum dots were found to be relatively nontoxic.

Introduction

The synthesis of nanocrystalline semiconductor materials with controlled sizes and biological compatibility are important in current material science research and has attracted a lot of interest in recent years.[1,2] Biological applications such as biosensing and cell imaging requires tuning the opto-electronic and surface properties, therefore making it essential to produce semiconductor nanocrystals with narrow size distributions in the 3−8 nm size region that are stable and water-soluble.[3,4] In recent years, a need for versatile and reliable imaging agents has stemmed from the fact that standard fluorescent labels such as organic dyes have been reported to be limiting, due to factors such as signal intensity strength, short lifetimes, and narrow excitation ranges.[5] Semiconductor nanocrystals have improved optical qualities desirable for biological applications compared to organic dyes, making their controlled synthesis and surface functionalization a very active area of research.[6−10]

Quantum dots are becoming popular as replacements for fluorescent dyes in biological fluorescence imaging because of their superior resistance to photobleaching. To date, considerable emphasis has been placed on using CdSe based quantum dots as biological chromophores, since they emit light that can be tuned throughout the visible spectrum.[11] However, concerns have been raised about the toxicological issue of quantum dots in living systems. Quantum dot toxicity can stem from two sources: (i) the quantum dot core and (ii) the capping molecule. A seminal study by Derfus et al. showed that quantum dots with a CdSe core and without a ZnS shell, after exposure to UV light, were toxic to liver cells.[12] The relatively low toxicity, and facile synthesis of photoluminescent germanium quantum dots make them ideal for biological fluorescence imaging and should eliminate any potential toxicology problems of quantum dots that might arise from having a CdSe core.[13−15]

Germanium nanocrystals are expected to exhibit photoluminescence in the visible region and quantum confinement effects due to the relatively large excitonic

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Bohr radius (R_b = 11.5 nm) and have been proposed for use in different applications because of their unique optical properties.[16,17] However, reports on germanium nanocrystals as biological tools/labels are few. For example, Boyle et al. reported the synthesis of ~3−5 nm phospholipid-encapsulated water-soluble germanium nanocrystals that were used as biomarkers for cell signaling in RBL-2H3 cells in vitro.[18] For germanium nanocrystals to be used in biological applications, photoluminescence quantum yield in the visible region and water solubility are vital. Thus the development of synthetic methods for producing germanium nanocrystals with controlled sizes and modified surfaces, with low toxicity, is important for the biological applications of germanium nanocrystals.

Germanium nanocrystals have previously been produced using different methods.[19−24] Veinot et al. produced alkyl-surface functionalized germanium nanocrystals via metal hydride reduction of nonpolar solutions of CTAB and GeI_4 at room temperature.[19] Kauzlarich et al. investigated the synthesis of germanium nanoparticles by the sodium naphthalide reduction of $GeCl_4$ varying reactions conditions.[20] Korgel et al. formed high yields of Ge nanocrystals by the reduction of GeI_4 by $LiAlH_4$ in high boiling point solvents.[21] Supercritical solvents have also been used to synthesize reasonable yields of germanium nanocrystals.[22−24]

The size controlled syntheses of monodisperse germanium nanocrystals using colloidal routes that involve high reaction temperature are well established. However, less progress has been made in the low temperature synthesis of Ge nanocrystals mainly because of its covalency.[25] The high temperature solution synthesis method to form Ge nanocrystals typically requires temperatures ranging from 270 °C to as high as 429 °C.[2,26] Such techniques, while useful in producing highly crystalline nanocrystals, are challenging, because of the instability of organic moieties such as solvents and capping agents at elevated temperatures which can generate hard to remove impurities. Additionally, interpretation of fluorescence data becomes difficult as reactive free radicals from the decomposition of organic solvents produce luminescence.[27]

Thus, while solution methods involving high temperatures are well established for germanium nanocrystals, room temperature syntheses that produce water-soluble, monodisperse germanium nanocrystals for cell imaging have not, to the best of our knowledge, been previously reported.

Here we show a simple method for the size controlled synthesis of photoluminescent germanium nanocrystals using reverse micelles and hydride reducing agents at room temperature. The hydride terminated nanocrystals were then surface stabilized and made water-soluble by capping with allylamine. The method reported here utilizes hydride reducing agents of different strengths to control nanoparticle size and produce hydrogen-terminated surfaces that could be used to react with compounds containing a C=C bond to produce different surface functionalities through the formation of a Ge−C surface bond.

The difference in reactivity of the different hydride reducing agents is used to control the sizes of nanocrystals formed in the reaction. Through controlling the size of the germanium nanocrystals, the photoluminescence properties of the quantum dots could be tuned.

Experimental Section

Synthesis and Purification. All of the chemicals used in this experiment were of analytical grade. In a typical synthesis, 50 mL of anhydrous hexane (Aldrich, ≥99%) was added to a 3-necked flask attached to a Schlenk line and purged with nitrogen. A total of 0.026 g of pentaethylene glycol monododecyl ether ($C_{12}E_5$), a nonionic surfactant (Nikko Chemicals Co, >98%), and 100 μL of $GeCl_4$ (Aldrich, ≥99.0%) was removed from the glovebox in an airtight syringe and injected into the reaction flask. Germanium nanocrystals were then formed by adding a stoichiometric amount (1:4, $GeCl_4$:reductant) of a solution of either $LiAlH_4$ (1.0 M in THF), $Li(Et)_3BH$ (1.0 M in THF), $LiBH_4$ (2.0 M in THF), or $NaBH_4$ (2.0 M in triethylene glycol dimethyl ether) as reducing agent and were all purchased from Aldrich Chemicals. After the above mixture was left to react for 2 h, the surface of the nanocrystals is terminated with hydrogen. A total of 40 μL of H_2PtCl_6 (99.995%, Aldrich) in anhydrous isopropanol (99.5%, Aldrich) as catalyst and 2 mL of allylamine (≥99%, Aldrich) were added to modify the surface germanium−hydrogen bonds, thus rendering the nanocrystals water-soluble.

The water-soluble Ge nanocrystals were purified by first removing the solvent by rotary evaporation to produce a yellow viscous oil. A total of 50 mL of distilled water was then added to the flask, in which the nanocrystals but not the $C_{12}E_5$ dissolved, which is subsequently removed by filtration. After sonication for 5 min the solution was further filtered using a Millipore 0.45 μm membrane filter. The filtrate was concentrated to about 1 mL and then purified down a Sephadex gel LH-20 (bead size 25−100 μm) column (φ = 1 cm). The luminescent fraction was then collected and concentrated down to 1 mL to give a solution of water-soluble germanium nanocrystals.

Characterization. Transmission electron microscopy images and electron diffraction patterns were acquired digitally with a JEOL 2010 operated at an accelerating voltage of 200 KeV. EDS data were acquired on a JEOL 2010 equipped with an Oxford Inca EDS detector. Photoluminescence spectra were obtained

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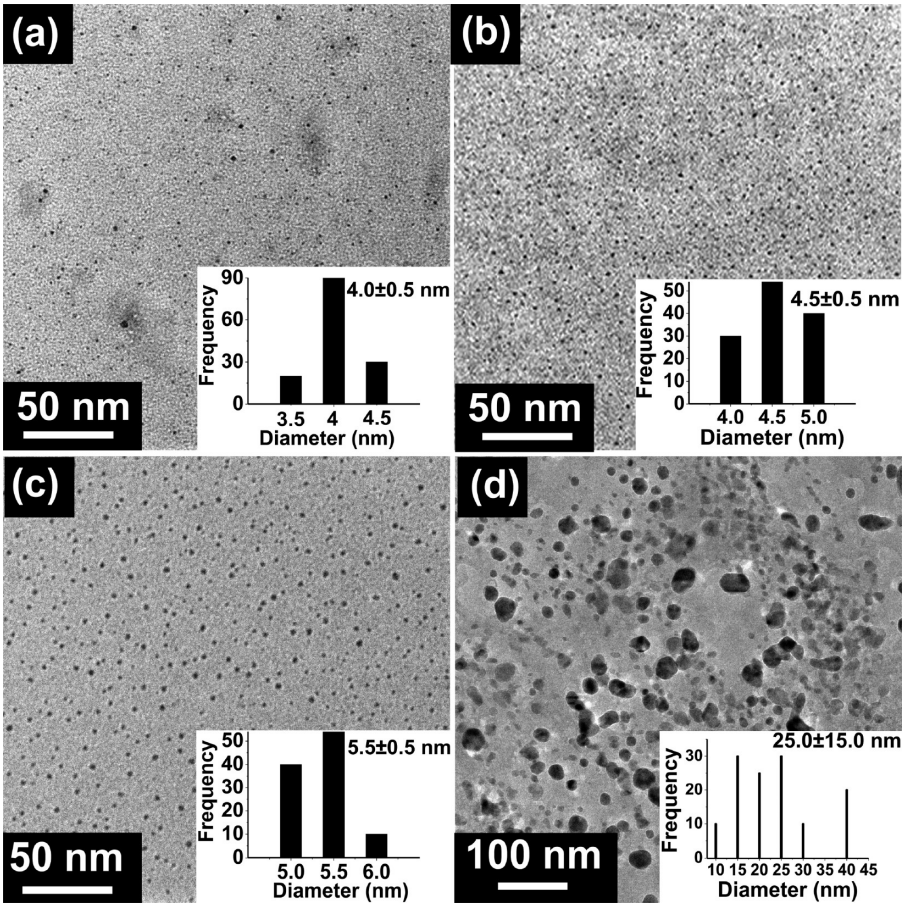

Figure 1. TEM images of Ge nanocrystals obtained from reduction by (a) $LiAlH_4$ and (b) $Li(C_2H_5)_3$, (c) $LiBH_4$, and (d) $NaBH_4$.

using a J-Y Fluorolog fluorescence spectrometer, with a slit width of 5 nm in both emission and excitation.

Incorporation of Ge Nanocrystals into HepG2 Cells. HepG2 cells were used with assays conducted at 1.0 μg/mL for 6 h of incubation. The germanium nanocrystals were excited with a mercury lamp, and the images were captured on a cooled charge-coupled device (CCD), mounted on a fluorescent microscope IX-81 (Olympus) with an excitation filter 330−380 nm.

Results and Discussion

It has been previously shown that by using reducing agents with different reactivities, the size of the resulting nanocrystals can be controlled.[20] To compare the effect of reductants in our reaction, four experiments were conducted. The first used $LiAlH_4$ as reductant, primarily because of its powerful reducing capability and also since it has been previously shown to produce nanocrystals of narrow size distribution.[28] Additionally $Li(C_2H_5)_3BH$ (superhydride), $LiBH_4$, and $NaBH_4$ were used with the strength of the reducing agents decreasing along this list with $LiAlH_4$ being the strongest and $NaBH_4$ the weakest. In this study, all other experimental variables were kept constant, and the samples were reacted for 3 h at room temperature, with the reducing agents added in one swift injection.

Figure 1 shows low-resolution transmission electron microscope (TEM) images of a number of allylamine capped germanium nanocrystals. The images illustrate that the selective use of the hydride reducing agents can control the size of the resulting Ge nanocrystals.

Figure 1a is low resolution TEM image of germanium nanocrystals obtained from the reduction using $LiAlH_4$. The nanocrystals had average sizes of 4.0 with a range of sizes of ±0.5 nm (based on 300 nanoparticles) and are predominantly spherical. Figure 1b,c are low resolution TEM images of germanium nanocrystals obtained from the reduction using $Li(Et)_3BH$ and $LiBH_4$, respectively. The nanoparticles were again found to be predominantly spherical and relatively monodisperse with average sizes of 4.5 nm and a range of sizes of ±0.5 nm (based on 500 particles) and 5.5 nm and a range of sizes of ±0.5 nm (based on 250 particles), respectively. Figure 1d is a low resolution TEM image of germanium nanocrystals obtained from the reduction using $NaBH_4$. The nanoparticles were found to be larger in size and were irregularly shaped nanocrystals with an average size of 25.0 ± 15.0 nm (based on 300 particles).

The reaction with $LiAlH_4$ produced the smallest nanocrystals (4.0 ± 0.5 nm) as shown in Figure 1a. The reaction using $LiAlH_4$ as the reducing agent proceeds vigorously and quickly due to the strong reducing capability of $LiAlH_4$. As a result of this rapid reduction there is a quick depletion of monomers with few monomers remaining for further growth of the nanocrystals, hence, the small particle size. A similar trend is observed for the

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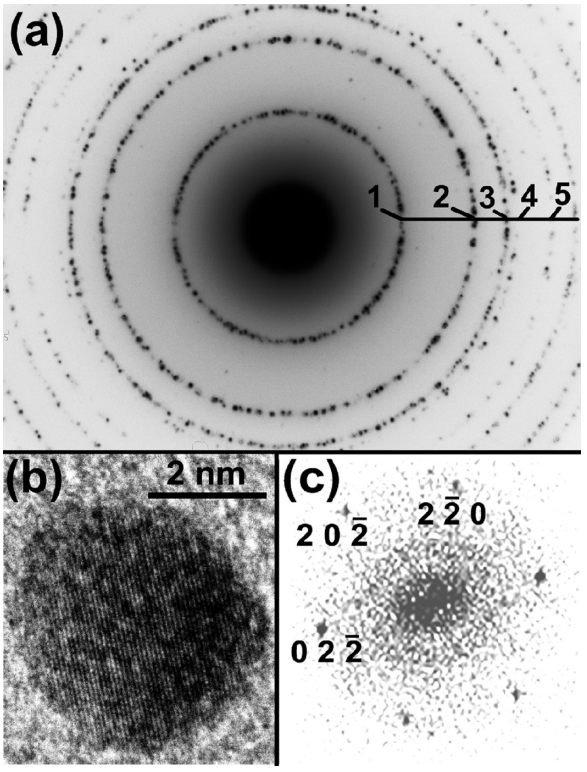

Figure 2. (a) Selected-area electron diffraction pattern (SAED) for Ge nanocrystals obtained from the hydride reduction of $GeCl_4$, rings matched the diamond germanium planes: Ring 1 = (111), 2 = (220), 3 = (311), 4 = (400), and 5 = (331), (b) high-resolution TEM image of an amine-terminated Ge nanocrystal synthesized using $LiAlH_4$ as reductant, viewed down the [111] direction (c) corresponding fast Fourier transform (FFT) of the image.

reduction by $Li(C_2H_5)BH$ and $LiBH_4$, but due to their lower reactivity compared to $LiAlH_4$, slightly larger nanoparticle sizes are obtained. $NaBH_4$ is a weak reducing agent, and when injected into the solution there is less monomer depletion in the nucleation burst leaving considerable unreacted monomer that contributes to nanoparticle growth, causing the formation of nanoparticles of large size.

The ability to control particle size in group IV nanocrystals using reducing agents is uncommon and to the best our knowledge not reported for systems involving hydride reducing agents and inverse micelles. Of particular significance is the ability to control nanoparticle size in the quantum confinement size regime.

Confirmation of the crystal structure of the germanium nanocrystals was obtained from selected-area electron diffraction (SAED) and is shown in Figure 2a. The SAED rings matched well with the diamond crystal structure adopted by germanium. Figure 2b is a high-resolution TEM image (HRTEM) of a 4 nm germanium nanocrystal obtained from the reaction using $LiAlH_4$ as reductant. The nanocrystal is highly crystalline with the lattice fringes clearly observable. The fast Fourier transform of the HRTEM image in Figure 2c exhibits the characteristic pattern of the diamond germanium crystal structure, viewed down the [111] direction. Crystalline nanocrystals of germanium such as these were obtained for all hydride reducing agents.

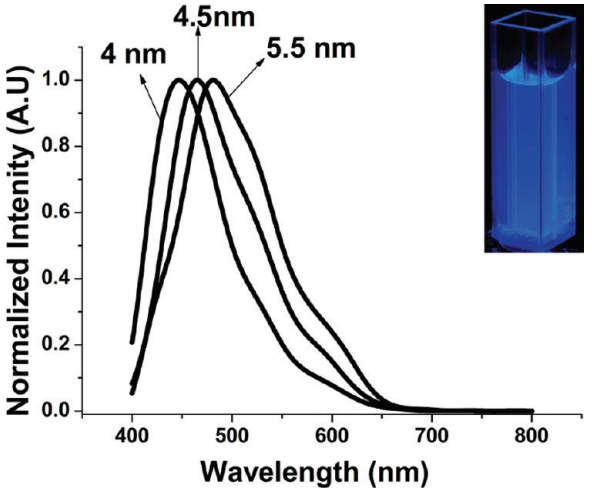

Figure 3. Photoluminescence spectra of amine-terminated Ge nanocrystals excited at 400 nm. The inset shows the fluorescence of the germanium nanocrystals excited under a UV lamp.

Figure 3 shows the 400 nm photoluminescence spectra (normalized) of an aqueous solution of allylamine-capped Ge nanocrystals of sizes 4.0, 4.5, and 5.5 nm made using $LiAlH_4$, $Li(C_2H_5)_3BH$ (superhydride), and $LiBH_4$, respectively. Emission across a relatively narrow region of 420−480 nm is observed with full width maxima (fwhm) of 70−80 nm indicating the monodisperse nature of the Ge nanocrystals. The PL peaks between 420 to 480 nm are similar to previous reports of Ge nanocrystals.[11,29] Quantum yield measurements based on the comparative method of Williams and co-workers relative to the standard 9,10- diphenylanthracene in cyclohexane indicated the germanium nanocrystals had a quantum yield of approximately 11%.[30−32]

As expected, no photoluminescence from direct band gap transitions was detected from the nanocrystals of average size 25 nm, which were too big to be in the quantum confinement regime. These results are important as they illustrate the ability to control nanoparticle size in the quantum confinement regime leading to the Ge nanocrystals exhibiting tunable photoluminescence in the visible wavelength region via direct band gap transitions.[33−36]

One application of germanium nanocrystals is as imaging agents for cell biology, and this application is demonstrated with HepG2 cells. Figure 4 shows the overlay of the transmission and fluorescence image obtained from HepG2 cells with Ge nanocrystals transfected into

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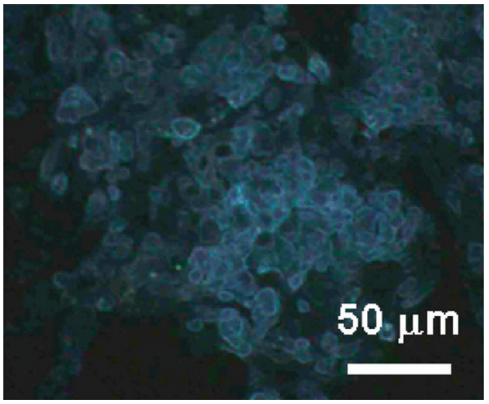

Figure 4. Overlay of the transmission and fluorescence microscopy image of HepG2 cells with germanium nanocrystals transfected inside the cytosol.

the cytosol. The blue fluorescence from direct-bandgap emission from the Ge nanocrystals in the HepG2 cells illustrates the use of hydrophilic germanium nanocrystals as biological fluorescence imaging agents.

To use germanium nanocrystals in biological imaging the effect on cell activity should be known. Mitochondrial activity in 0−1000 μg/mL of germanium nanocrystals was investigated with HePG2 cells as a carcinoma cell line. From the histogram in Figure 5, the 50%-inhibitory toxicity concentration (TC50) with HePG2 cells was calculated and found to be 100 μg/mL. This indicates that germanium nanocrystals could be used at concentrations of 100 μg/mL and have relatively low toxicity. A TC50 of 100 μg/mL is lower than that reported by Fujioka and co-workers for CdSe quantum dots exposed to UV light with HeLa cells.[37] This present study can provide the basis for future advances on the cytotoxicity of germanium nanoparticles.

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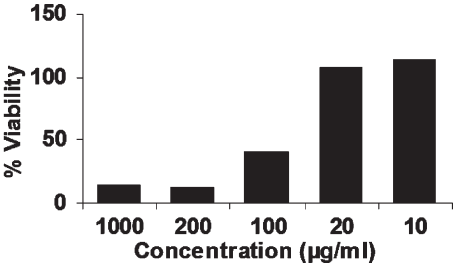

Figure 5. Toxicity studies: mitochondrial activity of HePG2 cells cultured with germanium nanocrystals.

Conclusions

In conclusion germanium nanocrystals with narrow size distributions were synthesized in reverse micelles and powerful reducing agents at room temperature and pressure. By using the difference in reactivity of the reducing agents, size control of the quantum dot was achieved. It was observed that by using $LiAlH_4$, the strongest reductant, small nanoparticles could be obtained, and by using weaker reductants such as $NaBH_4$, larger nanoparticles were obtained. The surface of the germanium nanocrystals were made water-soluble by capping with allylamine. The germanium nanocrystals were purified by column chromatography. The germanium nanocrystals dispersed in aqueous solvents displayed strong photoluminescence in the blue region of the visible spectrum. The amine functionalized germanium nanocrystals were used for the imaging of HePG2 cells and were shown to be relatively nontoxic. The optical properties, purity, and cell compatibility of the germanium nanocrystals reported here make them excellent candidates for future biological optical probes.

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