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Synthesis and characterization of a new water-soluble non-cytotoxic mito-tracker capped silicon quantum dot

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Allyl triphenylphosphonium bromide based mito-tracker capped silicon quantum dot (**Mito-SiQDs**) has been synthesized through an inverse micelle process. It was then fully characterized by transmission electron microscopy, energy-dispersive X-ray spectroscopy, dynamic light scattering techniques and X-ray photoelectron spectroscopic method. Energy dispersive X-ray spectroscopy analyses of the quantum dots confirm the presence of carbon, silicon, phosphorous and bromine atoms in **Mito-SiQDs**. Morphological study by transmission electron microscopy experiment showed the formation of the particles of size 11-12 nm of quantum dot dimension. The high negative zeta potential value of –23.7 mV calculated from dynamic light scattering study indicates the high stability of the circumvent agglomeration of **Mito-SiQDs**. The mito-tracker capped silicon quantum dot has blue emission at 400 nm wavelength upon excitation at 327 nm. **Mito-SiQDs** has not shown any significant cytotoxic effect with 10 to 50 µL/mL concentration on HeLa cell line for at least up to 12 h of its treatment. The **Mito-SiQDs** would be useful a possible fluorescent marker to visualize mitochondrial subcellular compartment in living cell through fluorescence imaging study.

Keywords: Silicon quantum dots, Fluorescence, XPS, Cytotoxicity, Mitochondrial target

A considerable attention has been paid to silicon quantum dots (SiQDs) and nanoparticles (SiNPs) by the scientific community over the last 20 years due to their distinctive activities in various aspects of novel optical, mechanical, electronic and catalytic properties.[1,2] Now, quantum dots (QDs) have acquired an incredible space of interest in the field of detection, biosensing, cellular imaging, drug delivery, therapeutic uses, optical marker in biomedical applications etc,[3-10] because of their remarkable photostability, good luminescence of narrow emission band and high fluorescent quantum yield. Again, silicon nanoparticles (SiNPs) are also significantly important as new fluorescent bioprobes considering their attractive advantages of controllable surface modification, inertness, abundant silicon source, high luminescence quantum yield, strong photostability, good bio-compatibility having relatively low toxicity.[11-14] So, the combination of these properties of SiQDs could generate a new horizon for bioimaging purposes as fluorescent cellular markers in a number of diagnostic tools and assays.[15,16] Moreover, optical property of SiNPs remarkably changes when its particle size is reduced to less than the bulk Bohr exciton radius (4.6 nm for silicon).[17,18]

Various physical and chemical methods including microemulsion technique,[19,20] sol-gel method,[21,22] electrochemical etching,[23,24] ultrasonic dispersion of electrochemically etched silicon,[25,26] thermal vaporization,[27] laser-induced pyrolysis of silane,[28,29] microwave irradiation methodology,[30-33] etc. are generally performed to synthesize SiQDs. Most of the synthetic methods follow either complicated procedures or drastic reaction conditions using hazardous reagents or using instrument with potential security. In this regard reverse micelles technique is good one,[34-37] but still in this procedure a variety of reducing agents has been used.[38,39] So, there is an increasing demand of acquiring a method of an easy-to-make SiQDs to explore the activity in biological system.

Recently, subcellular-targeted therapy is a promising area in biomedical nanotechnology for cancer treatment due to their higher efficacy with minimum side effect. The therapeutic results could be improved by many folds if pharmaceutical agents are

specifically and selectively directed toward the targeted organelle, i.e., pro-apoptotic compounds to mitochondria,[40,41] otherwise the random interaction of pharmaceuticals with intracellular site of action leads to the unwanted results. The mitochondrion is a double-membrane of phospholipid bilayer enveloped cytoplasmic organelle to have its own internal genome. The mitochondria of eukaryotic cells are the indispensable source of energy for survival and the translocation of proapoptotic proteins from the mitochondrial inter membrane space to the cytosol is regulated for controlling the activation of programmed cell death (so-called apoptosis) mechanism.[42] Thus, nowadays it's a huge challenge to the researchers to design mitochondria targeted pharmaceuticals and drug carriers considering the mitochondrion as an important therapeutic target in cancer therapy.[43-45] In this context SiQD is a good choice to employ for biomedical applications as it fulfils the essential criteria of water-solubility as well as hydrophilicity to prevent aggregation and precipitation in a biological environment with a considerable photoluminescence quantum yield in the visible region and a fast radiative recombination rate.[46]

Considering the above fact, herein we demonstrate a water-soluble allyl triphenylphosphonium bromide based mito-tracker capped silicon quantum dot (**Mito-SiQDs**) as a mitochondrial-targetable moiety which is easy to synthesize exploring a simple two-step procedure of high yield and selectivity at room-temperature. To the best of our knowledge so far, the step of functionalization to acquire the SiQDs is a new approach. The surface of QDs has been functionalized by triphenylphosphonium cation as water-soluble component and which can act as mitochondrial-targetable moiety. The synthesized isolated material has been characterized as **Mito-SiQDs** with the help of transmission electron microscopy (TEM), fluorescence spectrophotometry and X-ray photoelectron spectroscopy (XPS) analyses. The strong blue fluorescence (em: 400 nm) of this material was recorded under excitation of 327 nm in aqueous medium. **Mito-SiQDs** did not show any significant cytotoxic effect with 10 to 50 μL/mL concentration on this HeLa cell line for at least up to 12 h of its treatment.

Materials and Methods

All chemicals and reagents and spectroscopic grade solvents were purchased from Sigma. HITACHI F-7000 fluorescence spectrophotometer was used for the fluorescence spectra in all experiments. The morphological analysis and selected area electron diffraction (SAED) study of the synthesized Mito-SQDs was carried out by Jeol JEM 2100 HR with EELS transmission electron microscopy (TEM). XPS study was carried out by PHI 5000 Versa Prob II, FEI Inc.

Cell cytotoxicity assay

In order to study the cytotoxicity of **Mito-SiQDs**, 3-(4,5-dimethylthiazol-2-yl)-2,S-diphenyltetrazolium bromide (MTT) assay was carried out following the procedure described earlier. After the treatment of **Mito-SiQDs** (10, 20, 50, 100 and 200 μL/mL) for 6 h and 12 h, 10 μL of MTT solution (10 mg/mL PBS) was added in each well (96-well culture plate) and further incubated at 37 °C for 3 h at dark condition. Then, 100 μL of isopropyl alcohol (acidic) was added into each well after the removal of all media from wells. The produced formazan crystals (blue-violet) intracellularly were solubilized with 0.04 N acidic isopropyl alcohol. After that, the absorbance of the solution was recorded at wavelength 595 nm using a microplate reader (Model: THERMO MULTI SCAN EX). Three independent experiments were performed to calculate the mean. The cell cytotoxicity was calculated as % cell cytotoxicity = 100% – % cell viability.

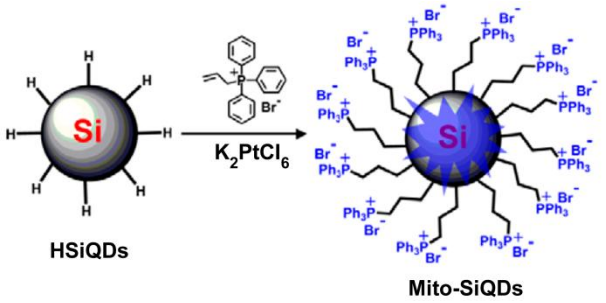
Synthesis of Mito-SiQDs

The target material (**Mito-SiQDs)** was prepared in two steps. In the first step, HSiQDs was synthesized following the previously reported synthetic method of silicon QDs by inverse micelle.[47-49] In the second step, a mixture of 1.0 mL 0.1 M methanolic solution of hexachloro-platinum and triphenyl-allyl-phosphonium bromide (50.0 mg) in 5.0 mL methanol solution was added to the methanolic solution of HSiQDs (20.0 mL) obtained from the first step using $SiCl_4$ (90.0 μL). The solution was stirred for 5 h in room temperature. Removal of solvents by a rotary evaporator led to the formation of white dry powder. The unreacted surfactant as impurity in the dry powder was removed through dispersion into 20 mL of Milli-Q water followed by sonication and then by consecutive filtration through a 0.22 μm Milipore. Express PES membrane and dialysis for 2 days in slide-A-Lyzer dialysis cassette G2, 2000 MWCO, 15 mL capacity to obtain the aqueous solution of **Mito-SiQDs** in pure form.

Results and Discussion

Synthesis and characterisation of Mito-SQDs

Synthesis of the material (**Mito-SiQDs)** involves two steps. In the first step HSiQDs from $SiCl_4$ following the literature method and in the second step conversion of HSiQDs to **Mito-SiQDs** was performed (Scheme 1). The second step is very important for the functionalization of the surface of HSQDs to produce **Mito-SiQDs** with the help of Chalk-Harrod hydrosilylation reaction with a modification[50] using K_2PtCl_6 as catalyst taking usual precaution for isolation. To the best of our knowledge so far, this step of functionalization is a new approach to obtain silicon quantum dots (SiQDs).



Scheme 1 — Synthetic strategy of **Mito-SiQDs**

Morphological analysis

Size and morphology of the synthesized **Mito-SiQDs** were confirmed from the TEM micrograph and DLS experiment. Fig. 1a shows the formation of the **Mito-SiQDs** with a size of 11-12 nm which are of quantum dot dimension. The selected area of electron diffraction (SAED) pattern (Fig. 1b) confirms highly crystalline nature of the QDs. Five bright concentric rings seen in the image are due to reflections from (111), (200), (220), (311) and (222) planes typical of the FCC lattice nature of the material.[52] Energy dispersive X-ray spectroscopy (EDS) analysis of the obtained mito-tracker capped silicon quantum dot shows that this compound contained carbon, silicon, phosphorous and bromine atoms.

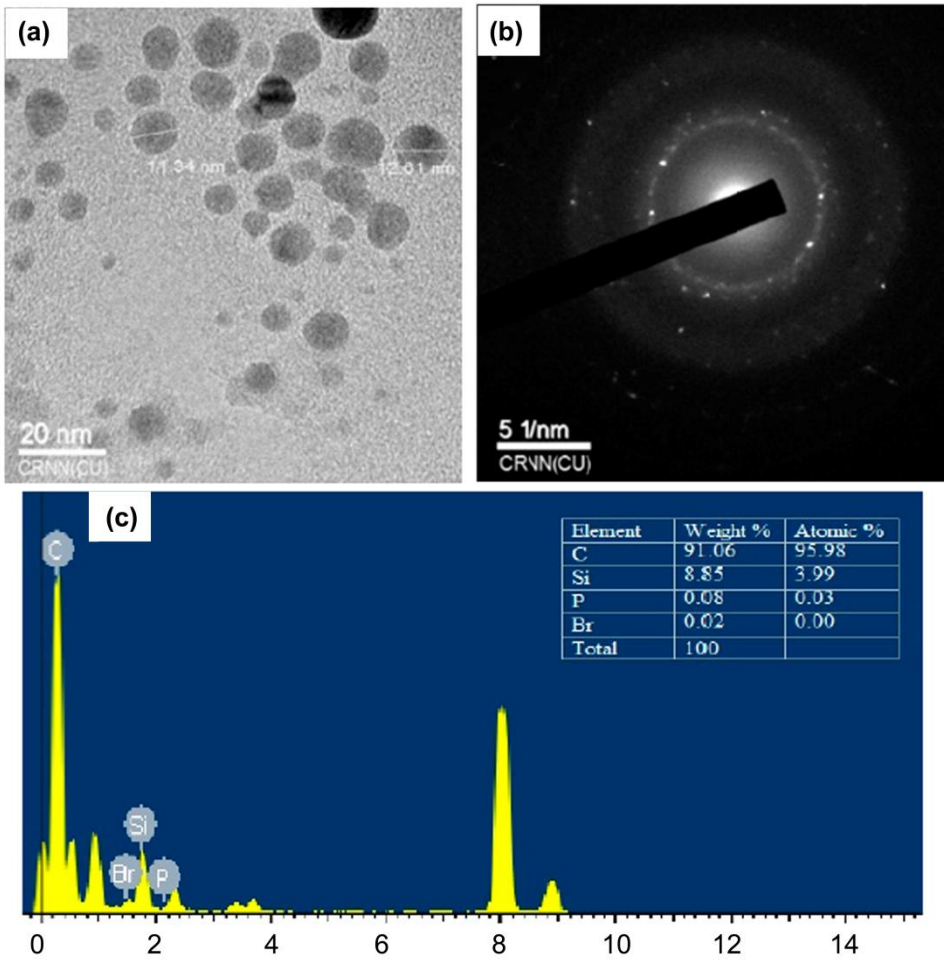


Fig. 1 — (a) TEM image, (b) SAED pattern and (c) EDS analysis spectrum of **Mito-SiQDs**

The size of particles obtained in the DLS may not coincide with the size obtained by TEM images as DLS measurement reveals the hydrodynamic radius of the particles along with the overall mobility of the entity. Hence, the size of **Mito-SiQDs** ~43 nm was measured by DLS measurement (Fig. 2), which is contrary to that observed by TEM (11-12 nm). Zeta potential value is an indication of the stability of the colloidal solution. The high negative potential of solution indicates high stability of this circumvent agglomeration. Experimentally, we have found that **Mito-SiQDs** has zeta potential of –23.7 mV (Fig. 3). This high negative potential indicates the formation of small nano-dimensional particles which are stabilized by allyl triphenylphosphonium bromide providing protection against precipitation. The low conductivity value of this material, 0.21 mS/cm represents the formation of nano-dimensional entities in this case because smaller particle size increases the overall charge density on the surface. This also enhances inter-particle repulsion as seen in the higher negative zeta potential value.

XPS analysis

XPS analysis of the prepared **Mito-SiQDs** showed that the binding energies of 284.96 eV, 284.95 eV, 287.44 eV and 283.75 eV corresponding to the C 1s of C-C, C-H, C-P and C-Si bonds of allylated silicon quantum dot, respectively. Furthermore, the binding energy of Si 2p at 100.56 eV indicated the presence of Si-C bond and 99.71 eV for Si-Si bond in the core. Binding energy at 70.22 eV revealed the presence of Br-P bond at the matrix (Fig. 4). All these findings

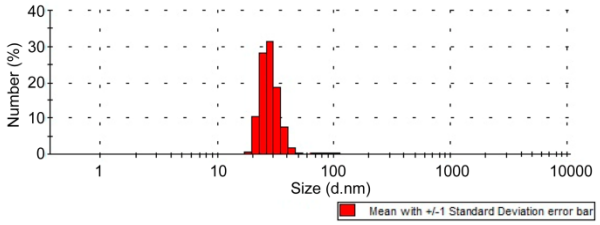


Fig. 2 — Dynamic light scattering spectrum displays the size and distribution of allyl triphenylphosphonium bromide-capped silicon nanoparticles in water

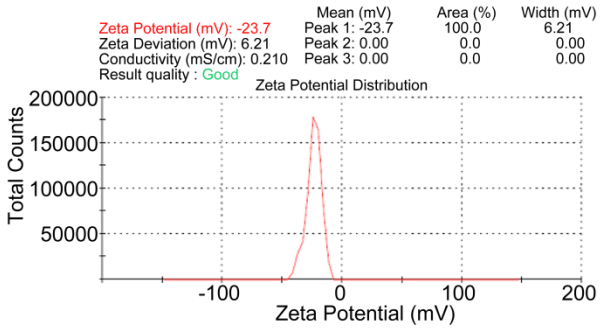


Fig. 3 — Zeta potential graph of **Mito-SiQDs**

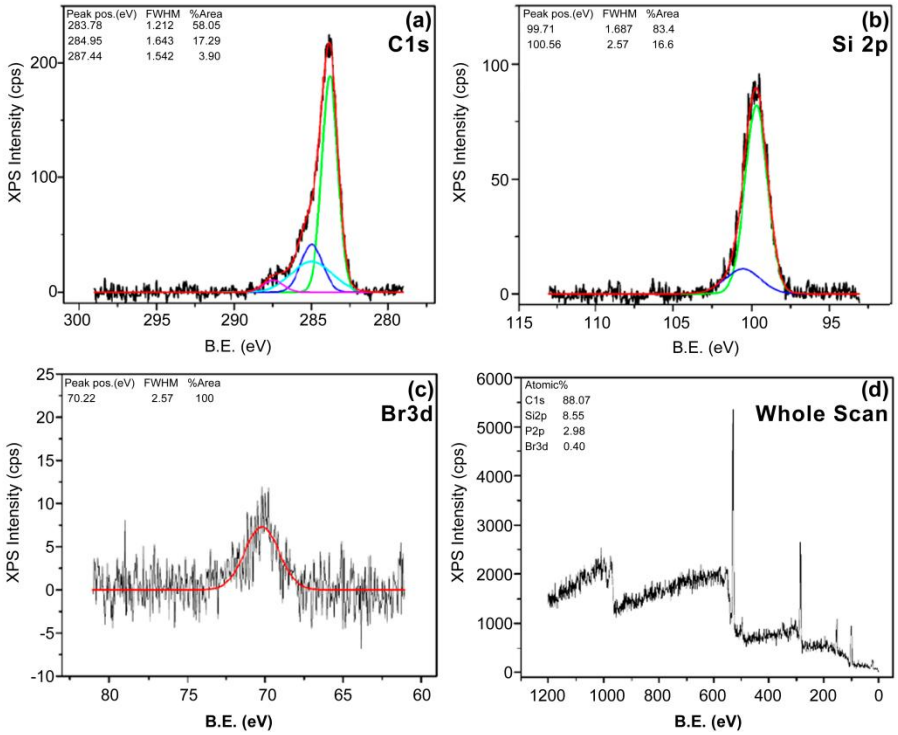


Fig. 4 — XPS core-level spectra of **Mito-SiQDs**. Dotted line is experimental data that is fitted with various mixed components. (a) C 1s, (b) Si 2p, (c) Br 3d and (d) total scan

proved the presence of allylated triphenylphosphino bromide in **Mito-SiQDs** and also the characteristic binding energy attributable to Si-C bonds confirmed the conversion of SiH_4 to allylated quantum dots. The individual binding energy peaks corresponding to Si-H 2p3/2 could not be differentiated in the XPS as the particles were coated with the allyl triphenyl phosphonium bromide. All data presented are from capped SiNPs, because "uncapped" SiNPs as such do not exist. Without any capping, bare silicon nanoparticles are very quickly oxidized under ambient conditions and are not biocompatible. However, the involvement of the quantum dot particle in stabilizing the material is clearly reflected by the presence of Br^- ions. These observations are quite in good consistent with literature.[53-57] Binding energies of various bonds of **Mito-SiQDs** calculated through XPS analysis were presented in Table 1.

Design strategy and fluorescence Study

Keeping in mind to target the mitochondria-subcellular region, we have attached triphenylphosphonium bromide moiety which is well known for its mitochondrial-targetable behaviour. Here, the triphenylphosphonium bromide group behaves as a 'targetable-engine' which may force to enter into a particular sub-cellular compartment in living cells when **Mito-SiQDs** will be introduced as a fluorescent marker. Excitation-emission spectra are an important tool for the characterization of quantum dots. Excitation spectrum was recorded from 260 to 390 nm to study the optical properties of **Mito-MSQDs** (10 µL/mL) (Fig. 5a). It showed a prominent sharp peak at around 327 nm. The appearance of the peak at 327 nm is the characteristic peak of **Mito-SiQDs**. Excitation spectra suggest the formation of highly stabilized **Mito-SiQDs**. For the emission study aqueous solution of this material was excited at 327 nm and spectrum was recorded from 350 to 650 nm at room temperature with emission maximum (λ_{max}) at 400 nm as presented in Fig. 5b. Observed spectrum is an agreement with the literature data in favour of the formation of quantum dots and this emission of the QDs occurred probably due to the jump of promoted conduction band electron to the valence band.[36,49,51,58] Thus, the fluorescent spectra revealed the successful formation of **Mito-SiQDs**. Theoretical literature report also suggests that 1-2 nm silicon quantum dots with a hydrogen or carbon surface termination have direct band gap for optical transitions that lead to photoluminescence in the blue region.[59]

Cytotoxicity

The bio-compatibility as well as cytotoxic effect of **Mito-SiQDs** in the living cells, was tested against breast adenocarcinoma HeLa cells using a standard method, the MTT assay. This material exhibited about 97, 95, 92, 88 and 82 % of cell viability at the concentration of 10, 20, 50, 100 and 200 µL/mL, respectively, when incubated for 6 h. Further, it showed about 95, 92, 90, 82 and 78 % of cell viability with the material concentration of 10, 20, 50, 100 and 200 µL/mL, respectively when the incubation time increased to 12 h (Fig. 6). However, significant cytotoxicity was noticed for higher doses (200 µL/mL) of **Mito-SiQDs** irrespective of the incubation period. Therefore, the **Mito-SiQDs** at a concentration of 10 to 50 µL/mL may be used safely for the purpose of fluorescence imaging of living cells.

Table 1 — Binding energies of various bonds of **Mito**

		Peak position			
C 1s	Bond	**C-C**	**C-H**	**C-P**	**C-Si**
	B.E. (eV)	284.96	284.95	287.44	283.78
Si 2p	Bond	**Si-C**	**Si-Si**		
	B.E. (eV)	100.56	99.71		
Br 3d	Bond	**Br-P**			
	B.E. (eV)	70.22			

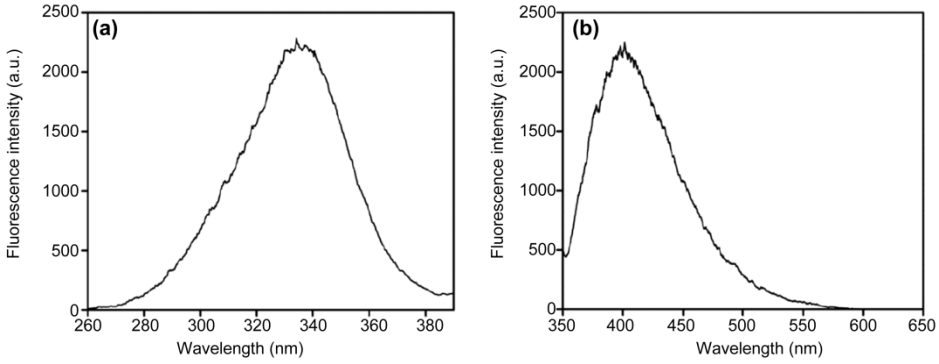


Fig. 5 — (a) Excitation and (b) emission spectra of **Mito-SiQDs** (10 µL/mL)

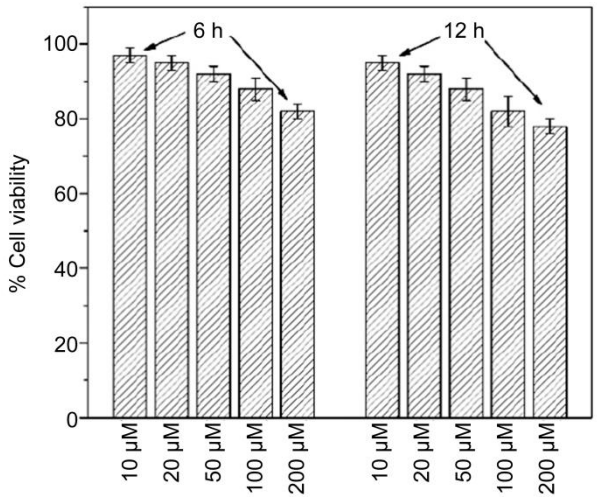


Fig. 6 — Cytotoxic data of **Mito-SiQDs** in breast adenocarcinoma HeLa cells incubated for 6 and 12 h

Conclusions

In summary, we have successfully prepared allyl triphenylphosphoniumbromide based quantum dots (**Mito-SiQDs**) through an inverse micelle process. It has been well characterized through various spectroscopic and morphological analyses. The strong blue fluorescence (em: 400 nm) of **Mito-SiQDs** was recorded under excitation of 327 nm in aqueous medium. This material did not show any significant cytotoxic effect with 10 to 50 µL/mL concentration on this HeLa cell line for at least up to 12 h of its treatment. The **Mito-SiQDs** could be useful a possible fluorescent marker to visualize mitochondrial subcellular compartment in living cell through fluorescence imaging study.

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