Synthesis of Si/SiO$_2$ core/shell fluorescent submicron-spheres for monitoring the accumulation of colloidal silica during the growth of diatom *Chaetoceros* sp.

Luu Manh Quynh*, Hoang Van Huy$, Nguyen Duy Thien$, Le Thi Cam Vanb, Le Viet Dungb

*Center for Materials Science, Faculty of Physics, VNU – University of Science – 334 Nguyen Trai, Thanh Xuan, Hanoi 100000, Vietnam
$Faculty of Fisheries, Vietnam National University of Agriculture – Trau Quy, Gia Lam, Hanoi 100000, Vietnam

**Abstract**

Marine diatoms play a very crucial role in carbon export, and current food-web and become an important factor in global silica cycle. This has made the mechanism of their biosilicification interesting to be a research subject. The classical theory states that the silica metabolism has been originated from the absorption of silicate ions, which might not give a suitable explanation for the solid silica silicification. In this study, mono-disperse Si/SiO$_2$ fluorescent submicron-spheres were synthesized in aqueous solution, and applied in monitoring the extracellular solid silica accumulation of *Chaetoceros sp*., diatom. The Si/SiO$_2$ submicron particles emitted light-blue color with the spectrum centered at 440 nm under the excitation of 365 nm UV light, similar to the typical excitation/emission pair of the DAPI fluorophore (excitation/emission: 358 nm/461 nm). The fluorescence-microscopic investigation showed that the Si/SiO$_2$ particles delocalized on the diatoms’ surface and increased a silicic-acid-level surrounding the microalgae. As a consequence, the growth rate of the diatoms increased as the concentration of the SiO$_2$ particles was at 120 mg/L, and reached 1.5 times higher than the growth rate calculated from the F$_2$ media. The study not only introduces a new aspect to the extracellular metabolism of microalgae biosilicification corresponding to the global silica cycle, but also presents a new-type of cultivating media using SiO$_2$ nanoparticles for diatom cultivation, which increases the growth rate of artificial diatom-culturing for further applications.

**Keywords:** Si Quantum dots; SiO$_2$ submicron-spheres; diatom cultivation; extracellular biosilicification; silica cycle

1. **Introduction**

Being responsible for 40% of the oceanic primary productivities, diatoms are of the most population contributors to global phytoplankton, leading them to play a significant part of a core source of carbon export [1], current marine foodwebs [2] and silica cycle [3]. Their abundant fatty acid and protein have expanded their bioactivities aiding in aquaculture feeding system [4,5], human health and cosmetic [6,7]. Besides, their frustule secondary-structural silica cell wall is functioned to create different porous silica-configurations in nanoscale with high surface area, which are promising candidates for any extended applications in modern technologies, such as wastewater treatment [8], drug delivery [9,10], biosensing [11,12] and energy production[13].

In view of the above mentioned magnificent values, diatoms have been isolated and cultivated intensively [14,15]. Based on the cultivating mediums as found by Provasoli [16] and Guillard [17], additional nutrients have been added to enrich individual element content [18,19] or to investigate the growth rate [19,20] of diatoms. Silica-containing chemicals, commonly being Na$_2$SiO$_3$ in classical cultivating media [17-20], would be the indispensable nutrient-feeding components recommended in diatom cultivating media. As a consequence, increasing the silica consumption is required for the growth rate during a diatom cultivation process. However, the concentration of silicate ions is generally bounded under 100µM [17-19,21]. At higher concentration, the silicate ions might precipitate with other nutrients, such as Fe$^{3+}$, Co$^{2+}$ and Cu$^{2+}$, present in the cultivating media, leading to the lack of metal nutrients. Roseline et al. showed that the elevating concentration of silica in culturing media - up to 2 mM - indicated the low growth rate and smaller size of the diatoms [20]. Hence, further increasing the growth rate of the diatom cultivation has been challenging.

By a symbiosis cultivation process with a coccolithophor, Okcu et al. presented a method to increase the silica consumption in the culturing solution to improve the diatom growth rate [22]. In the article, under rich CO$_2$ condition, the NO$_3^-$ formed during the coccolithophor development reacted with the Si(OH)$_3$ in solution, created colloidal SiO$_2$ and indicated the elevating growth of the diatoms [22]. In another study under a scanning microscope, Ikeda found out that the colloidal SiO$_2$ particles at sub-micron sizes accumulated on the surface of the diatoms [23]. The author suggested that the small size of colloidal SiO$_2$ could assist the development of the
marine microalgae. Besides, a new insight of biosilicification has also been proposed by Ikeda in two steps: extracellular silica absorption and intracellular silica cell-wall deposition. The extracellular metabolism agrees with the classical theory of diatom silicification in which the absorbed SiO2(OH)2 2- ions are transported to silica deposition vesicles (SDV) where the silica polymerization process and exocytosis to cell wall occur [24]. The classical theory states that the extracellular silicic acids in the form of Si(OH)4 2- ions are absorbed through the cell wall by a silicic-acid receptors (Si-receptors) [24,25]. In contrast to this, Ikeda recommended that the silicic acid in the form of Si(OH)2 formed colloidal SiO2 particles, immobilized on diatoms’ surface before being absorbed into the cell [23]. This suggestion explained the presence of colloidal SiO2 on the diatom surface, but did not clarify the transportation of colloidal SiO2 through the cell wall and becoming intracellular SiO2(OH)2 2- ions.

The localization of SiO2 nanoparticles could be monitored under a fluorescent microscope by implanting organic [26] or inorganic [27] fluorophores into the particles. Of the fluorophores implanted SiO2 particles, owing to their high quantum luminosity and stability, quantum dots / SiO2 particles in core/shell structures become the important candidates as potential probes for bioimaging [27]. Belonging to these core/shell systems, biocompatible Si/SiO2 core/shell nanoparticles were synthesized by a simple hydrothermal process emitting the light-blue color at around 450 nm under 360 nm excitation light [28]. These excitation-emission wavelengths were close to those of the typical dye in biology 4,6-diamidino-2-phenylindone (DAPI – excitation/emission: 358 nm/461 nm). Therefore, they were sufficient for monitoring the solid silica extracellular delocalization on diatom under microscope.

In this work, aiming to study the extracellular metabolism of colloidal SiO2, luminescent submicron SiO2 spheres were synthesized and monitored the delocalization of luminescent sub-micron silica particles on the surface of the diatom under a fluorescent microscope during the growing process of Chaetoceros sp. With this, the role of submicron colloidal SiO2 particles as silica-consumption for diatom growth was investigated as well.

2. Materials and Methods

2.1. Synthesis of Si/SiO2 submicron-spheres

Si/SiO2 submicron-spheres were synthesized through two steps: (i) the preparation of Si quantum dots by a hydrothermal process and (ii) coating the quantum dots with a SiO2 submicron spherical cover. In the first step, the typical hydrothermal process of Si quantum dots synthesis has been described elsewhere by Zhong [29]. In detail, 3 mL of (3-aminopropyl) triethoxysilane (APTES – MERCK CAS 919-30-2) was added into a three-neck beaker that contained 1 g trisodium citrate (TSC – MERCK CAS 6132-04-3) diluted in 30 mL distill water. The solution was stirred for 10 min before being transferred to a Teflon autoclave. It then was sealed in a stainless steel cover for a 12h hydrothermal process at 170°C. After the hydrothermal process, the byproducts from the reaction were filtered by a 3.5 kDa cellulose dialysis tube under vigorous stirring at 4°C in 24 h. The product as-received was labeled as “Si QDs”. In the second step, the SiO2 coating of the Si QDs was carried out following a modified-Stober hydrolysis of tetratetlyl orthosilicate (TEOS – MERCK CAS 78-10-4) [30]. Typically, 5 mL homogenous solution containing 7.4 mg TSC, 1 mL ammonium hydroxide solution 25% (Sigma Aldrich CAS 1336-21-6) and 3.75 mL Si QDs solution from 1st step was pipetted into 20 mL mixture of 50/50 methanol/ethanol. After 5 min., 2.5 mL TEOS was added and kept under vigorous stirring for 4 h. The product was washed by centrifugation, collected in aqueous solution and labeled as “Si/SiO2 spheres” before any investigations.

The second step was applied for the synthesis of SiO2 submicron spheres without the Si QDs at the core. The SiO2 particles as-received had the same size and shape with those of the Si/SiO2 spheres (data not showed); and later were used in diatom growth.

The X-ray diffraction (XRD), Fourier transformed infrared (FTIR) spectra, photoluminescence (PL) and scanning electron micrographs (SEM) of the Si QDs and SiO2 spheres containing samples were investigated respectively by Bruker D5005 - Siemens, Jasco FT/IR 4700 – Horiba, Prolog 3 – Horiba spectrometers and a Nova nanoSEM 450 – FEI electron-microscope at Center for Materials Science, Faculty of Physics, VNU – Hanoi University of Science. The transmitted electron microscopic (TEM) image of the Si QDs was observed by a FEI Tecnai transmission electron microscope (TEM) at Institute of Physics, Vietnam Academy of Science and Technology.

2.2. Culturing media preparation for Chaetoceros sp. culturing

Chaetoceros sp. species was purchased from the Aquaculture Research Institute III, Nha Trang and was checked for its size, shape and concentration under a Neubauer counting chamber. All chemicals (shown in Table 1) were purchased from MERCK in analytical grade for synthesis. The Na+ added compositions NaNO3 (CAS 7631-99-4), NaH2PO4 (CAS 10049-21-5) and Na2EDTA,2H2O (CAS 6381-92-6); metals containing the compositions of FeCl3,6H2O (CAS 10025-77-1), CuSO4.5H2O (CAS 7758-99-8), ZnSO4.5H2O (CAS 7446-20-0), CoCl2.6H2O (CAS 7791-13-1), MnCl2.4H2O (CAS 7773-01-5) and Na2MoO4.2H2O (CAS 10102-40-6); the vitamins Thiamin.HCl (CAS 67-03-8), biotin (CAS 58-85-5) and B12 (CAS 68-19-9) in reagent grade. The culturing media for the diatom cultivation was prepared following the F2 published by Guillard [17] with the modification on silica source. Four culturing media were applied to investigate the role of solid-silica concentration on the development of the diatoms during cultivation. Conventional F2 was the first culturing media as a control test. In other three media, the silica source Na2SiO3 was substituted by different amount of submicron sphere silica with different concentrations labeled as 15SiO2, 30SiO2 and 120SiO2 corresponding to the added 15 mg, 30 mg and 120 mg of solid-silica SiO2 (Table 1). After vigorous stirring with no aggregation was experienced, 1.2 × 106 cells/mL Chaetoceros sp. diatoms were added into the media. The ambient temperature, illumination, air supply conditions were kept the same during the diatom growth. pH value of the diatoms containing growth solution was monitored throughout the
culturating progress and the same indoor-culturating installation was reported elsewhere [31].

| Composition                  | Conventional F2 | 15SiO$_2$ | 30SiO$_2$ | 120SiO$_2$ |
|------------------------------|-----------------|-----------|-----------|------------|
| Silica source                |                 |           |           |            |
| Na$_2$SiO$_3$                | 30 mg (107 μmol Si) | 0 | 0 | 0 |
| SiO$_2$ spheres              | 15 mg (250 μmol Si) | 30 mg (500 μmol Si) | 120 mg (250 μmol Si) | |
| Na$^+$ added composition     |                 |           |           |            |
| NaNO$_3$                     | 150 mg (1.675 mmol) | | | |
| NaH$_2$PO$_4$                | 10 mg (72.5 μmol) | | | |
| Na$_2$EDTA.2H$_2$O           | 8.7 mg (23.4 μmol) | | | |
| Metals and trace metals      |                 |           |           |            |
| FeCl$_2$.6H$_2$O             | 6.3 mg (23.3 μmol) | | | |
| CuSO$_4$.5H$_2$O             | 0.0196 mg (0.079 μmol) | | | |
| ZnSO$_4$.5H$_2$O             | 0.044 mg (0.153 μmol) | | | |
| CoCl$_2$.6H$_2$O             | 0.020 mg (0.085 μmol) | | | |
| MnCl$_2$.4H$_2$O             | 0.360 mg (1.830 μmol) | | | |
| Na$_2$MoO$_4$.2H$_2$O        | 0.0126 mg (0.052 μmol) | | | |
| Vitamins                     |                 |           |           |            |
| Thiamin. HCl                 | 0.2 mg | | | |
| Biotin                       | 1.0 μg | | | |
| B$_{12}$                     | 1.0 μg | | | |

2.3. Monitoring the delocalization of Si/SiO$_2$ submicron-spheres during the growth of diatom

The silica extracellular absorption of the Chaetoceros sp. diatoms during cultivation was obtained under the fluorescent microscope with the excitation/emission filters of DAPI (excitation/emission: 358 nm/461 nm). 30 mg of Si/SiO$_2$ – about 500 μmol of Si – was added in the F2 culturing media in the substitution of Na$_2$SiO$_3$ (same amount with the 30SiO$_2$ media). 0.5 mL diatoms containing samples right after the diatoms being added, and after 1 h, 2 h, and 4 h growing they were collected directly from the culturing beaker and had the microscopic images observed under an AXIO SCOPE 3, Carl Zeiss fluorescent microscope.

3. Results and Discussion

Figure 1 presents the XRD patterns and FTIR spectra of the Si QDs and Si/SiO$_2$ spheres containing powders. The XRD pattern of Si QDs exhibited a broad peak centered at 21.1° corresponding to the amorphous phase of the particles [28,33]. On the FTIR investigation, the characteristic vibrations of APTES attributing to the N–H wagging arose at 758 cm$^{-1}$, 1484 cm$^{-1}$ and 2881 cm$^{-1}$ in the spectrum of Si QDs and disappeared in the spectrum of Si/SiO$_2$ spheres corresponding to the coating effect of the SiO$_2$ cover. Besides, the strong IR absorption at 1064 cm$^{-1}$, 1024 cm$^{-1}$ related to the Si–O–Si stretching vibration of APTES molecules agreed with earlier published studies [26,32,33].

The Si QDs had the size of 40 nm (Figure 2A), and were mostly in spherical shape in which 4 nm Si nanocrystals were covered by SiO$_2$ layer (Figure 2B). This structure was representative to the structure of the Si QDs synthesized by hydrothermal method [29,33] where the Si-O linkages from the APTES molecules were reduced to Si seed by TCS under the high pressure of saturated vapor at 170°C in the autoclave. After the formation of the Si seed, silane groups from the APTES were immobilized on the Si surface and formed Si-O stable cover, followed by the bonding of the 3-aminopropyl tails (-C$_3$H$_7$-NH$_2$) [26,33]. As a result, the N–H bonds still existed in the Si QDs, as discussed above by the FTIR in figure 1.
study of Ikeda [23], which would be convenient for an investigation of the solid silica extracellular metabolism of the diatoms.

To study the microalgae biosilicification by fluorescent microscope, the photoluminescence of the Si/SiO$_2$ was investigated. Under the excitation of ultraviolet light, the particles emitted light-blue color with the spectra centered at 440 nm (Figure 3). As the excitation wavelength changed from 250 nm to 400 nm, the fluorescent intensity at 440 nm was measured and the photoluminescence excitation (PL excitation) spectrum was achieved. As shown in figure 3, this spectrum shows the excitation wavelength of 365 nm where the highest luminescent intensity is being observed. The photoluminescence of the Si/SiO$_2$ is similar to the one in the Si QDs obtained by other authors [27,32] witnessing that the Si QDs are successfully covered into the SiO$_2$ coat.

The diatom growth is required silica in soluble phase [17-19]. As the silicate ions (SiO$_4^{2-}$) have been added to the media, they accumulate on the surface of the diatom and form SiO$_2$(OH)$_2^{2-}$ formula to become suitable for the diatom to absorb through a silica-receptor on their surface [24,25]. Therefore, the diatoms might release chemicals that can elevate the pH of the solution, as also experienced in our culturing progress in which the pH value increased from 7 to 8.5. The basis condition (high pH value) created a corrosion effect on the SiO$_2$ through the reaction (2).

$$\text{SiO}_2 + 2\text{OH}^− \rightarrow \text{SiO}_2^{2−} + \text{H}_2\text{O} \rightarrow \text{SiO}_4(\text{OH})_2^{2−} \quad (2)$$

The presence of the silicic acid on the Si/SiO$_2$ spheres’ surface led to the migration of the particles to the Si-receptor on the diatoms, making the diatom illuminated under the UV excitation. After 1 h cultivation, most Chaetoceros sp. cells were covered by Si/SiO$_2$ spheres (figure 4B$_1$). By this process, aqueous silica was released from solid silica from Si/SiO$_2$ spheres and then diluted into the media indicating the diatom growth. After 2 h and 4 h, the concentration of the diatoms increased by their division; hence, it was possible to view less fluorescent diatoms under a microscope (figure 4C$_2$, D$_2$).

Figure 5 shows the SEM of the Si/SiO$_2$ particles collected from the culturing media. It was observed that before interaction with Chaetoceros sp. cells in solution, the surface of the particles was smooth. After 1 h and 2 h in media, the surface of the particles became rough. Some crushed spikes visualized on the particles indicated that the particles were corroded (figure 5B, C). At 4 h growth of the diatom, blur effect could be seen on the SEM image of the Si/SiO$_2$ spheres that might correspond to an organic layer covering onto the particles, which may be the presence of the released chemical from the diatoms (figure 5D). The similar blur effect of SEM image on organic layer coated nanoparticles was observed by Tetsu’s group [37]. The organic coat created an electron backscattering effect, which made the SEM imaging become difficult. As consequence, blur effect might occur.

The delocalization of the SiO$_2$ particles on diatom-surface was also observed by Ikeda [23]. In this published article, the SiO$_2$ grains with the size in the range of 200 nm to 800 nm were
achieved by transmission electron microscopic (TEM) imaging, those attached on the surface of marine diatoms. The author also stated that this immobilization of the solid silica plays a very important role in biosilification of the global silica cycle. The diatoms migrate to the larger solid silica sources such as soils or silica mine, and cause corrosion to increase aqueous silica level in natural water. After the biosilification, silica in solid phase is accumulated in the diatom cell wall; hence with ocean currents; it would be dispersed into the ecosystem.

\[
\ln \frac{C_t}{C_0} = \ln \left( \frac{1}{t \ln 2} \right) \tag{3}
\]

following the earlier studies [17,19,20], where the \(C_t\) and \(C_0\) are the concentration measured at times \(t\) and 0 respectively. Figure 7B demonstrates the dependence of as-calculated growth rates of the diatoms under in-this-study customized culturing media. The growth rates were experienced to decrease corresponding to the decrease of the relative media concentration in comparison with the concentration of the diatoms. From another perspective, the growth rate increased as the added amount of the \(\text{SiO}_2\) particles increased, and reached 1.5 times higher than the growth rate calculated from the F2 media.

Some recent articles show that the growth rate of the diatom in solution did not increase as much during the elevation of the silica concentration [20,21,23]. In those studies, the added silica was in aqueous phase – in the form of silicate ion or of silicic acid. As we discussed above, at that high concentration those ions might create aggregation with the trace metals in

Fig. 6. Micrograph (A) and absorption spectrum at [550 nm – 850 nm] region of the Chaetoceros sp. containing sample at \(6.52 \times 10^6\) cells/mL concentration. The dependence of diatom concentration on the absorbance at 685 nm (C) and the correlation between the concentrations measured by spectrophotometer and by microscope at the range from 1.2\(\times10^6\) to \(6\times10^6\) cell/mL (D).

Figure 7 illustrates the change of the Chaetoceros sp. concentration during 4 days cultivation in different culturing media. The initial concentration of the diatoms in all the culturing process was \(1.2 \times 10^6\) cells/mL. In conventional F2 media, the algae grew fast in the first 2 days, reached the maximum population after 3 days and started the death phase in the 4th day. This result agreed with the earlier result published by Tokushima [41]. By adding 15 mg, 30 mg and 120 mg of \(\text{SiO}_2\) submicron-spheres, the molar concentration of the Si atom were about 2.5 times, 5 times and 20 times higher than that of in F2 media, respectively (see in Table 1); the diatoms grew faster. The growth rate in form of number divisions per day of the algae was calculated by the formula

\[
d = \ln \frac{C_t}{C_0} \left( \frac{1}{t \ln 2} \right) \tag{3}
\]

in F2 media. The growth rates in form of number divisions per day of the algae were calculated by the formula

\[
d = \ln \frac{C_t}{C_0} \left( \frac{1}{t \ln 2} \right) \tag{3}
\]
solution, and consequently cause the lack of nutrient to the media; hence, it might decrease the growth rate of the microalgae.

Taking into account the silicic acid release from solid silica initated by the diatoms, the high surface area of the SiO₂ nanoparticles not only increase the aqueous silica level in the culturing media, but also play as silica slow-release agent that indicates the high concentration of the silicic acid on the diatom surface. This is followed by the growth of the Chaetoceros sp. in solution. Therefore, the nanosize SiO₂ particles can be a good candidate for silica source for marine diatom cultivation, and might pave an appropriate way to develop the culturing media for higher growth rate diatom-accumulation.

4. Conclusion

To sum up, 720 nm mono-disperse Si/SiO₂ fluorescent submicron-spheres with narrow size-distribution were successfully synthesized in aqueous solution. Under the 356 nm light excitation, submicron particles emitted light-blue color with the spectrum centered at 440 nm, which could be monitored under a fluorescent microscope via the typical mode of the DAPI fluorophore for an investigation of extracellular biosilicification of Chaetoceros sp. The Si/SiO₂ particles migrated to the surface of the diatoms released silica in silicic acid form and being transported into the microalgae. This process created high concentration of aqueous silica at the diatoms’ surface. As a consequence, the growth rate increased as the concentration of the SiO₂ particles was 120 mg/L, and reached 1.5 times higher than the growth rate calculated from the F2 media. The study not only presents a new-type of culturing media using SiO₂ nanoparticles for diatom cultivation, but also introduces a new aspect to the extracellular metabolism of microalgae biosilicification.

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