Synthesis of highly stable cyanine-dye-doped silica nanoparticle for biological applications

To cite this article: Ying Lian et al 2018 Methods Appl. Fluoresc. 6 034002

View the article online for updates and enhancements.

Related content

- Fluorescent proteins as efficient tools for evaluating the surface PEGylation of silica nanoparticles
  Wei Zhang, Minyan Ma, Xiaoai Zhang et al.

- Multivalent linkers for improved covalent binding of oligonucleotides to dye-doped silica nanoparticles
  S M Kelleher, R I Nooney, S P Flynn et al.

- Bifunctional nanoparticles with magnetism and NIR fluorescence: controlled synthesis from combination of AGET ATRP and ‘click’ reaction
  Weiwei He, Liang Cheng, Lifen Zhang et al.
Synthesis of highly stable cyanine-dye-doped silica nanoparticle for biological applications

Ying Lian, Long-Jiang Ding, Wei Zhang, Xiao-ai Zhang, Ying-Lu Zhang, Zhen-zhen Lin and Xu-dong Wang

Department of Chemistry, Fudan University, 200433 Shanghai, People’s Republic of China

E-mail: wangxudong@fudan.edu.cn

Keywords: cyanine dye, silica nanoparticles, reverse micelle method, Stöber methods, stability

Abstract

Cyanine dyes are widely used in biological labeling and imaging because of their narrow near infrared emission, good brightness and high flexibility in functionalization, which not only enables multiplex analysis and multi-color imaging, but also greatly reduces autofluorescence from biological matter and increases signal-to-noise ratio. Unfortunately, their poor chemical- and photo-stability strongly limits their applications. The incorporation of cyanine dyes in silica nanoparticles provides a solution to the problem. On one hand, the incorporation of cyanine dyes in silica matrix can enhance their chemical- and photo-stability and increase brightness of the nanomaterials. On the other hand, silica matrix provides an optimized condition to host the dye, which helps to maintain their fluorescent properties during application. In addition, the well-established silane technique provides numerous functionalities for diverse applications. However, commercially available cyanine dyes are not very stable at high alkaline conditions, which will gradually lose their fluorescence over time. Our results showed that cyanine dyes are very vulnerable in the reverse micelle system, in which they will lose their fluorescence in less than half an hour. The existence of surfactant could greatly promote degradation of cyanine dyes. Fluorescent silica nanoparticles cannot be obtained at the high alkaline condition with the existence of surfactant. In contrast, the cyanine dyes are relatively stable in Stöber media. Owing to the fast formation of silica particles in Stöber media, the exposure time of cyanine dye in alkaline solution was greatly reduced, and highly fluorescent particles with good morphology and size distribution could be obtained via Stöber approach. However, the increasing water content in the Stöber could reduce the stability of cyanine dyes, which should be avoided. This research here provides a clear guidance on how to successfully synthesize cyanine dye-doped silica nanoparticles with good morphology, size distribution, stability and brightness.

1. Introduction

Fluorophore-doped silica nanoparticles have received tremendous attention in the last decades, not only because they have outstanding optical properties and biocompatibility, but also because they offer endless possibilities for further functionality using well-established silane technique [1–4]. The advanced technologies in precisely tuning their morphology and size endow them as ideal materials for biological studies [5–8]. Therefore, it is not surprising to witness the wide applications of fluorophore-doped silica nanoparticles in the fields of biological labeling [4, 9, 10], in-vivo imaging [11, 12], luminescence sensing [13, 14], theranostic, clinical diagnosis [15] and many other areas [16]. The broad choices of available fluorophores that covers a wide range of electromagnetic spectrum give fluorophore-doped silica materials the ability of multiplex analysis and multi-color labelling and imaging [17]. One of the leading biological application of fluorophore-doped silica nanoparticles is in-vivo imaging. For this particular application, luminescent silica nanomaterials that emit in the near-infrared (NIR) region, especially near the biological window, are highly favored, simply because these materials have a high background-to-noise
ratio, reduced autofluorescence and deep tissue penetration depth.

In the class of NIR-emitting fluorophores, cyanine dyes are particularly interesting because they possess narrow emission band, which makes multi-color imaging possible. More attractively, the emission spectrum of cyanine dyes can be tuned by extending the π-conjugated system in their chemical structures. The resulting derivatives show diverse spectroscopic properties with absorbance ranging from UV/Vis to infrared (700 to 1100 nm) [18]. The side chain of the heterocyclic rings of cyanine dyes can be modified to possess new functional groups for further functionalization. More importantly, cyanine dyes exhibit large molar extinction coefficient (typically at 200 000 L mol⁻¹ cm⁻¹) [19] and good brightness. Although cyanine dyes offer attractive features, their poor chemical- and photo-stability limited their applications. In addition, bare cyanine dyes are not suitable to be directly used for biological labelling and imaging, because their large π-conjugated system can be easily attacked by reactive species inside cells, and the uncontrollable distribution inside cells puts great challenges on image processing and data interpretation. Therefore, cyanine dyes are usually encapsulated in or covalently linked with a certain matrix to form nanosized particles for fluorescence labelling and imaging [20–22]. The matrix not only protects the dye from irreversible damage but also provides a microenvironment to host the dye and stabilizes its luminescent properties. In addition, the matrix offers more sites for multiple functionalities [3]. silica is one of the promising matrix candidates to host cyanine dyes since they can be synthesized at mild conditions, and they offer attractive features in functionalities, physicochemical properties and excellent biocompatibility [11]. More importantly, it has been reported that incorporation of cyanine dyes into silica nanoparticles could tremendously improve the photo-stability and brightness of cyanine dyes [23]. In search of cyanine dye-doped silica nanoparticles, to the best of our knowledge, there are only few reports on the success of encapsulating cyanine dyes inside silica nanoparticles [24–32]. Within these reports, either more stable dyes [24, 26, 28, 30, 31] or biomaterials [29] were used to assistant the formation of fluorescent silica nanoparticles. However, commercially available cyanine dyes are very challenging to be incorporated into silica nanoparticles, mainly because these dyes cannot tolerant the alkaline condition used in silica nanoparticle formation [24, 27].

There are mainly two approaches for synthesizing silica nanoparticles: one is the template approach, which forms silica particles in a water-in-oil emulsion (also called reverse micelle). silica nanoparticles formed in this media have been well known for their better morphology and size control [33]. Alternatively, silica nanoparticles can be easily synthesized via the classic Stöber approach. silica nanoparticle can be massively produced with relatively good quality control via this route. However, particles with the diameter smaller than 100 nm does not have uniform size and round shape, which will influence their biological applications. one outstanding feature of Stöber method, which makes it so attractive, is that it is highly diversified and silica particles can be formed in quite different conditions. Recent research has revealed that silica nanoparticle with relatively uniform size can be formed using amino acid or phosphate buffer as catalyst [34–39]. In such mild condition, cyanine dyes are quite stable and can be successfully incorporated into silica nanoparticles [40]. These cyanine-doped silica nanoparticles have been widely used in biological applications and even been approved by the Food and Drug Administration in the USA for medical applications [41–43].

In this article, we have systematically studied the stability of cyanine dye in the synthetic media of silica nanoparticle formation, including the water-in-oil emulsion approach and the classic Stöber method, both of which have been widely employed for synthesizing dye-doped silica nanoparticle. We were surprised to observe that commercial-available cyanine dyes are not stable in the water-in-oil emulsion. There is a quick color change within half an hour, in concomitant with complete loss of fluorescence. Fluorescent silica nanoparticles cannot be obtained in the common reverse micelle approach. A modified reverse micelle approach was developed which can well preserve the fluorescence of cyanine dyes, but the morphology of obtained nanoparticles is not satisfied. We have proposed a new model to explain the poor stability of cyanine dyes in reverse micelle system. In contrary, highly fluorescent and stable silica nanoparticles can be formed via the classic Stöber method. However, the reaction media should be carefully chosen since it strongly influences the stability of cyanine dyes during particle synthesis.

2. Materials and methods

2.1. Materials
Cyanine5.5-N-hydroxysulfosuccinimide ester (Cy5.5-NHS, 90%, Purchase No. 2136026) and sodium fluoride (429323) were purchased from J&K Scientific Ltd (www.jkchemical.com). Aminopropyltriethoxysilane (APTES, A0439), cyclohexane (C0469), 1-hexanol (H0130) and triethylamine (TEA, T0424) were obtained from Tokyo Chemicals Industry Co. Ltd (www.tcichemicals.com). Tetraethyl orthosilicate (TEOS, 131903), Triton X-100 (T9284) and pluronic F-127 (P2443) were bought from Sigma-Aldrich (www.sigmaaldrich.com). Ammonia solution (28%, A-99960) was purchased from Heowns (www.heowns.com). Dried dimethyl sulfoxide (DMSO, 75927), absolute ethanol (01101143) and acetone (01194780) were purchased from Tansoole (www.tansoole.com).
2.2. Synthesis of CySiNPs via Stöber method

The synthesis was following the classic method reported by Stöber [44]. Typically, a concentrated ammonia solution (28%, 500 μl) was added to absolute ethanol (8.5 ml), followed by adding Cy5.5-NHS solution (25 μl) and TEOS (350 μl) in sequence. The mixture was stirred for 24 h at room temperature. The formed CySiNPs were harvested by centrifuging and washed three times using 95% ethanol to remove unreacted chemicals. The obtained CySiNPs were then redispersed in absolute ethanol at a concentration of 10.0 mg ml⁻¹.

2.2.3. Synthesis of CySiNPs via reversible micelle method

The reverse micelle (water in oil) media was formed by mixing cyclohexane (7.5 ml), 1-hexanol (1.8 ml), Triton X-100 (1.8 ml) and water (480 μl) with vigorous magnetic stirring [45]. To the mixture, a 28% ammonia solution (100 μl) was added and stirred for 10 min. Then, TEOS (100 μl) was added and the mixture was further stirred at room temperature for 30 min, followed by adding the Cy5.5-APTES solution (25 μl). After stirring for 24 h, acetone (10 ml) was used to break the reverse micelle. The precipitate was centrifuged at 17 000 g for 10 min, and the obtained nanoparticles were washed three times with 95% ethanol to remove surfactant and unreacted materials. Finally, the obtained nanoparticles were dispersed in absolute ethanol at a concentration of 10.0 mg mL⁻¹.

The synthesis of CySiNPs via a modified reverse micelle approach was conducted in a similar way, only the ammonia catalyst was replaced by sodium fluoride solution (20 mg ml⁻¹, 250 μl).

2.3. Influences of the chemical composition in the reaction media on Cy5.5-NHS stability

2.3.1. In reverse micelle reaction media

To study the influence of chemical components on the stability of cyanine dyes, cyanine dyes are exposed in different chemical environments as the list in table 1. The color change and the UV/Vis absorption spectrum of the reaction solution were recorded over time. All reagents were added according to the material composition in reversible micelle method. Cy5.5-NHS in DMSO (25 mM) was added to the different solutions respectively, and these solutions are named as sample S1-S8.

All chemicals were used as received without further purification.

---

### Table 1. Cy5.5-NHS in different solution (reversible micelle system).

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Cy5.5-NHS (μL)</th>
<th>Cyclohexane (μL)</th>
<th>1-hexanol (μL)</th>
<th>Water (μL)</th>
<th>28% Ammonia (μL)</th>
<th>Triton X-100 (μL)</th>
<th>Phuronic F-127 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>2.5</td>
<td>/</td>
<td>/</td>
<td>48</td>
<td>10</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>S2</td>
<td>2.5</td>
<td>1110</td>
<td>/</td>
<td>48</td>
<td>10</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>S3</td>
<td>2.5</td>
<td>930</td>
<td>180</td>
<td>48</td>
<td>10</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>S4</td>
<td>2.5</td>
<td>930</td>
<td>/</td>
<td>48</td>
<td>10</td>
<td>180</td>
<td>/</td>
</tr>
<tr>
<td>S5</td>
<td>2.5</td>
<td>750</td>
<td>180</td>
<td>48</td>
<td>10</td>
<td>180</td>
<td>/</td>
</tr>
<tr>
<td>S6</td>
<td>2.5</td>
<td>940</td>
<td>/</td>
<td>48</td>
<td>/</td>
<td>180</td>
<td>/</td>
</tr>
<tr>
<td>S7</td>
<td>2.5</td>
<td>760</td>
<td>180</td>
<td>48</td>
<td>/</td>
<td>180</td>
<td>/</td>
</tr>
<tr>
<td>S8</td>
<td>2.5</td>
<td>930</td>
<td>/</td>
<td>48</td>
<td>10</td>
<td>/</td>
<td>0.192</td>
</tr>
</tbody>
</table>

### Table 2. The effect of surfactants in reversible micelle system.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Cy5.5-NHS (μL)</th>
<th>Cyclohexane (μL)</th>
<th>1-hexanol (μL)</th>
<th>Water (μL)</th>
<th>28% Ammonia (μL)</th>
<th>Triton X-100 (μL)</th>
<th>TEOS (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S9</td>
<td>2.5</td>
<td>750</td>
<td>180</td>
<td>48</td>
<td>10</td>
<td>180</td>
<td>10</td>
</tr>
<tr>
<td>S10</td>
<td>2.5</td>
<td>930</td>
<td>180</td>
<td>48</td>
<td>10</td>
<td>/</td>
<td>10</td>
</tr>
</tbody>
</table>

### Table 3. The effect of basicity in Stöber system.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Cy5.5-NHS (μL)</th>
<th>Absolute ethanol (μL)</th>
<th>28% Ammonia (μL)</th>
<th>Water (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S11</td>
<td>2.5</td>
<td>1600</td>
<td>100</td>
<td>/</td>
</tr>
<tr>
<td>S12</td>
<td>2.5</td>
<td>2100</td>
<td>100</td>
<td>500</td>
</tr>
</tbody>
</table>
2.3.2. Study the effect of surfactants in reversible micelle media

From the experiments list in table 1, we observed that both concentrated ammonia and surfactant strongly influence the stability of cyanine dyes in the reverse micelle system. We have further designed experiments to elucidate the origin of the instability of cyanine dyes with or without the existent of surfactant. Cy5.5-NHS in DMSO (25 mM) was added to the different solutions as listed in table 2.

2.3.3. In Stöber system

The classic reaction media was formed by mixing absolute ethanol, ammonia and TEOS according to the report by Stöber. Later on, water was introduced into Stöber system in order to better control the size of obtained silica nanoparticles. We have studied the stability of cyanine dyes in both Stöber system with and without water. The color and the UV–vis absorption spectrum were recorded over time.

2.4. Instruments and characterization

The UV/Vis absorption spectra were recorded on a Hitachi U3900H UV–vis spectrometer. The color of solutions was captured using a digital single-lens reflex camera (Nikon D300s). The size, distribution and morphology of the synthesized CySiNPs were characterized using a transmission electron microscope (JEM 2011 operated at 200 kV). The quantum yields and lifetimes of samples were measured on an FLS980 fluorescence spectrometer (Edinburgh Instruments, UK). The dynamic light scattering measurement was conducted on a Malvern Zetasizer (Nano ZS, Malvern Instruments, UK).

3. Results and discussion

Cyanine dyes have been widely used for fluorescent labels [46], optical nanosensors [47], photographic sensitizers, biological monitoring [31] and imaging [48] due to their long emission wavelength, deep tissue penetration depth, large molar extinction coefficient, minimum photo-damage to biological samples and versatile in functionalization. However, their poor chemical stability and photostability have largely limited their applications. The incorporation of fluorescent dyes into silica nanoparticles has been proved to be an efficient approach to protect fluorescent dyes from decomposition. A large number of dyes can be incorporated inside silica matrix which not only improves dye stability but also leads to a significant increase in the brightness of nanomaterial [32]. Therefore, great progress has been made in preparing dye-doped silica nanoparticles. Wu et al synthesized a cyanine-derivative-doped silica nanoparticle, which exhibits extraordinary photo-stability and fluorescent brightness [25]. The CySiNPs were successfully applied in in vitro cellular imaging and in vivo imaging of a tumor-bearing mouse. Miletto and co-workers incorporated a cyanine dye in silica matrix to give fluorescent nanoparticles which exhibited nearly 1000-fold brightness enhancement compared to free dye in solution [28]. Thus, synthesizing CySiNPs could provide a promising platform for better utilization of cyanine dyes in various fields.

Our first attempt was to synthesize CySiNPs via the reversible micelle method, where ammonia was used as catalyst to promote hydrolysis of silanes. Nanoparticles produced via the reversible micelle method have been proved to have uniform shape, smaller size (within 100 nm) and narrow size distribution [49]. The size and size distribution could be
precisely controlled by adjusting the water to surfactant molar ratio \[\frac{50}{50}\]. We selected the commercial-available dye Cy5.5 as a representative cyanine dye, which has a molar extinction coefficient in dimethyl sulfoxide of \(138300 \text{ L mol}^{-1} \text{ cm}^{-1}\). However, our attempt to synthesize CySiNPs was completely failed, and we observed that the blue color of Cy5.5-NHS dye was completely changed into light yellow color (insert in figure 1(a)) within half an hour. The resulted nanoparticles have no fluorescence at all (figure S1, supporting information is available online at stacks.iop.org/MAF/6/034002/mmedia). We also tried other commercial-available cyanine dyes, including Cy3-NHS, Cy3.5-NHS and Cy5-NHS, the same phenomenon was observed for all the dyes (data not shown). All the tested cyanine dyes will change their original color into light yellow color and completely lose their fluorescence properties. This observation contradicts with the facts that several reports have successfully incorporated cyanine dyes inside silica nanoparticles via the reverse micelle approach \[25,26,28,32\].

Due to the fact that cyanine dyes are not stable in harsh conditions \[51\], we have designed different experimental conditions to study the stability of Cy5.5-NHS dye in these media. The experiment conditions were listed in tables 1, 2, and results are summarized in figure 2. It is obvious to see that the color of S4 and S5 (containing both concentrated ammonia and the surfactant Triton X-100) changed from their original blue to light yellow in less than 30 min. However, samples S1–S3, S6 and S7 containing either concentrated ammonia only or Triton X-100 only do not change their color, which means the dye is relatively stable in these media. In addition, the color of sample S1–S3, S6 and S7 can stay in blue for more than a week. From these observations, we can confirm that both the concentrated ammonia and surfactant have a great influence on the stability of cyanine dyes. Results from the UV–visible absorption spectrum of sample S5 at different time were shown in figure 2(B), which confirmed the degradation of cyanine dye in the reaction media. The absorbance of Cy5.5-NHS in the wavelength range between 625 and 750 was gradually decreased over time. Within half an hour, the absorbance of sample S5 at 700 nm decreased about 95%. In contrast, the color and UV–vis absorption spectrum of sample S3 without the surfactant were not changed during the observation time (figure S2 in the supporting information).

Based on these results, we proposed that the existence of both the surfactant and concentrated ammonia strongly influences the stability of cyanine dyes.

Figure 2. (A) The pictures of solution S1 to S8 as listed in table 1 (taken at 30 min). (B) UV/Vis absorption spectra of solution S5 at different time. Inset of B: the corresponding pictures of solution S5 taken the same time interval.
The concentrated ammonia in the reaction media will hydrolyze into free hydroxide ions. The hydroxide ions can act as strong electron donors and attack the electrophilic sites of cyanine structure [51], leading to slow destruction of π-conjugated system and disappearance of typical absorbance at 700 nm. However, the existence of surfactant significantly accelerates this process. Due to the unique chemical structure of cyanine dyes, they tend to distribute nearby the boundary of oil and water interface where the surfactant is located. The hydroxide ion can easily attack dyes distributed in this region and lead to the fast decomposition of cyanine dyes. This can be proved by changing the surfactant Triton X-100 into another non-ionic surfactant Pluronic F-127 (Sample S8). The same phenomenon was observed when both Pluronic F-127 and concentrated ammonia exist in the reaction media, and the cyanine dyes quickly lost their original color.

From the above results, we can conclude that the commercial-available cyanine dyes are not stable in the typical reverse micelle system, which contains both the non-ionic surfactant and concentrated ammonia to assist the formation of uniform silica nanoparticle. In order to form CySiNPs, an obvious solution is avoiding or reducing the exposure of cyanine dyes to highly alkaline condition during particle synthesis. It had been reported that the fluoride ion can catalyze the hydrolysis and condensation of organic silane, and has been used as the catalyst to form silica nanoparticle [52, 53]. With the aim of synthesizing CySiNPs with a uniform size in the reverse micelle media, we had tried to use sodium fluoride as catalyst instead of ammonia. The use of sodium fluoride solution greatly reduces the pH of reaction media, and the blue color of Cy5.5-NHS was well preserved during particle synthesis. CySiNPs were successfully synthesized via this method without destructing the conjugation structure of cyanine dyes. The obtained nanoparticle have strong blue color (inset of figure 1(b)) and a large absorbance at 700 nm in UV/Vis spectrum (figure 1(b)). Unfortunately, the morphology of obtained nanoparticle is not satisfied as shown in the TEM image (figure S3 in the supporting information). The diameter of synthesized CySiNPs was about 100 nm with poor size distribution.

Since fluorescent CySiNPs with uniform size and morphology cannot be synthesized in the typical reverse micelle approach, we tried to use the classical Stöber method to synthesize CySiNPs. Stöber method is a very simple, easy-to-operate, and low-cost method to quantitatively produce silica particles. More attractively, Stöber method has been well-established to synthesize particles with different sizes at various condition [54]. Silica nanoparticles can be formed in very mild conditions. Even amino acid can be used as basic catalysis to synthesize silica nanoparticles [55], which is a favorite condition for cyanine dyes. In addition, particles formed very quickly in the Stöber method, which can reduce the exposure time of the cyanine dyes to the alkaline media. Bringley et al [27] has synthesized cyanine-based nanoparticles using a modified Stöber method. They reduced the reaction time to several hours to decrease the exposure time of cyanine dyes to the highly alkaline condition. Though the obtained nanoparticles showed quiet high extinction coefficients and quantum yield, the shortened nucleation time leads to an irregular shape.

In order to develop a synthetic approach that could not only preserve the fluorescence of cyanine dyes but also maintain the physiochemical properties of nanoparticles, we first studied the stability of Cy5.5-NHS in the synthetic condition used in the Stöber method. As shown in figure 3(A), the dye is stable and keep their blue color for more than a week. From the UV–vis absorbance spectra, it is clearly shown that the dye is much more stable than in the reverse micelle system. There is only a slight decrease in absorbance at the first two days. Due to the fast formation of silica particle in the Stöber method, the stability of cyanine dyes in the synthetic media is long enough to form fluorescent CySiNPs with uniform size and morphology. However, the dye is not stable over long-term exposure. Once the solution was kept for 18 days (inset in figure 3(A)), the color of the solution changed into yellow and the dye loses its fluorescence. During the experiments, we also observed that water concentration in the synthetic media plays an important role in the stability of the dye. As shown in figure 3(B), once water was introduced into the synthetic media (Sample S12), the decomposition of the dye is accelerated, which is also confirmed by the changes of UV–vis absorbance. This is mainly because the introduction of water molecules could lead to the hydrolysis of ammonia, and produce more hydroxide ions in the media (figure S4 in the supporting information), which is harmful to the stability of cyanine dyes. Results suggested that fluorescent CySiNPs with uniform size and morphology could be synthesized via the Stöber method without the existence of water. The synthesized CySiNPs have regular shapes and a uniform size of ~48 nm (figure 4(A)). Dynamic light scattering results show that the CySiNPs have a polydispersity index of 0.086. The fluorescence quantum yields and lifetimes of free dyes and dye incorporated inside silica particles are characterized. The free dye in dimethyl sulfoxide has a quantum yield of 7.3%, and dye immobilized inside silica nanoparticle has a quantum yield of 6.0%. The lifetimes of dye before and after silica incorporation is 1.16 ns and 2.47 ns, respectively. Considering that a single silica nanoparticle contains multiple dyes, the extinction coefficient of dye inside CySiNPs was also measured, which shows a value of 2520 L g⁻¹ cm⁻¹. Thus, the strong luminescence (figure 4(B)) and good stability make the CySiNPs suitable for biological applications.
In summary, in order to successfully synthesize cyanine dye-doped silica nanoparticles with good morphology and size distribution, we have systematically studied the stability of cyanine dyes in both the reverse micelle system and Stöber system. Our results showed that commercial-available cyanine dyes are not stable in reverse micelle system. They will be decomposed in less than half an hour and accomplished with an obvious color change and complete loss of fluorescence. This instability was originated from the hydroxide ion in the synthetic media which will attack electrophilic sites of cyanine structure, leading to a slow destruction of the $\pi$-conjugated system and loss of fluorescence. The existence of non-ionic surfactants, such as Triton X–100 and Pluronic F127 will significantly accelerate this process, which induces the fast degradation of the dye in reverse micelle system. The solution to this problem is either synthesizing highly stable cyanine dyes (which is time- and labor-consuming) or reducing the exposure of dye in high alkaline media. The use of neutral pH catalyst sodium fluoride can form fluorescent CySiNPs, but the morphology and size distribution of obtained particles are not satisfying. In contrast, the classic Stöber approach is the method of choice to synthesize fluorescent CySiNPs with good morphology and size distribution, mainly because of better stability of cyanine dyes in the media and greatly reduced exposure time during particle formation. However, the increase of water content in the media will significantly reduce the stability of cyanine dyes, which should be taken into considerations during particle synthesis. The results shown here could give

![Figure 3](image1.png)

**Figure 3.** Photos under sunlight (insert) and UV/Vis absorption spectra of solution (A) S11 and (B) S12 (sample composition list in table 3) at different days as marked.

![Figure 4](image2.png)

**Figure 4.** (A) A transmission electron microscopic image of CySiNPs prepared via Stöber method. (B) Absorbance (black) and fluorescence (red) spectrum of CySiNPs prepared via Stöber method (excitation wavelength = 625 nm). Inset: a picture of the suspension of synthesized CySiNPs.
researchers clear guidance on how to synthesize cyanine-based nanoparticles with good stability and bright fluorescence. Attention should be paid on choosing the right reaction condition.

Acknowledgments

This work was financially supported by National Key R&D Program of China (2017YFC0906800), National Natural Science Foundation of China (21775029), the Recruitment Program of Global Experts (1000 Talent program) in China, and the Program for Professor of Special Appointment (Eastern Scholar) at Shanghai Institutes of Higher Learning (No. TP2014004), which are greatly acknowledged.

ORCID iDs

Xu-dong Wang https://orcid.org/0000-0002-3402-7995

References

[31] Accomasso L et al Fluorescent silica nanoparticles improve optical imaging of stem cells allowing direct discrimination between live and early-stage apoptotic cells Small 2012 8 3192–200


[44] Stöber W, Fink A and Bohn E 1968 Controlled growth of monodisperse silica spheres in the micror size range J. Colloid Interface Sci. 26 62–9


