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## Fluorescent Carbon by Covalently Attaching a BODIPY Fluorophore

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5 This work reports the preparation and characterization of fluorescent carbon particles by covalently attaching a BODIPY fluorophore and demonstrates the feasibility of using fluorescent carbon particle for visualization in micro/nanofluidics.

10 Fluorescent carbon particles may be valuable for many fields of science due to their superior chemical stability, non-toxicity, and environmental compatibility.<sup>1,2</sup> For example, these carbon particles have shown great potential in bioimaging and biolabeling.<sup>3</sup> Of particular interest, carbon particles with bright  
15 fluorescence are important in visualization of the movement of carbon particles in micro/nanofluidics.<sup>4</sup> As the development of “Labs on a Chip”, the interaction between particles and the wall of the nanoscaled channels is of great interest.<sup>4</sup> Polymer and metal particles have been studied. Carbon has a unique dielectric  
20 constant and density compared to polymers and metal. However, the study of carbon particles is limited because carbon does not fluoresce and is difficult to visualize.

The synthesis of carbon nanoparticles with bright fluorescence and low water solubility is challenging. Carbon nanoparticles  
25 with small diameter, < 5 nm<sup>1</sup>, are described as carbon dots (C-dots). C-dots have been synthesized by various methods such as candle soot method,<sup>5</sup> laser induced pyrolysis of hydrocarbons,<sup>6</sup> etc. Although C-dots that were passivated with polymers or organic molecules, or oxidized by oxidizing reagents exhibit high  
30 fluorescence quantum yield,<sup>6</sup> in most cases, the C-dots with strong fluorescence are soluble in water and cannot be used as fluorescent tracers.

Herein, we report the first fluorescent carbon particles by chemically attaching fluorophores on the surface. The  
35 fluorophore modified carbon particles exhibit bright fluorescence for micro/nanofluidics study. The prepared fluorescent carbon particles show significant advantages. The fluorescent carbon particles exhibit bright fluorescence and low solubility in water. Potentially the fluorescence quantum yield could be easily tuned  
40 by the linker distance and the choice of the molecular fluorophores. Moreover, the carbon particles with various sizes can be easily prepared and tested. Herein we show the preparation and characterization of fluorescent carbon particles with size of 5 μm and 100 nm by covalently attaching a fluorophore. 4, 4-difluoro-5, 7-dimethyl-4-bora-3a, 4a-diaza-s-indacene (BODIPY)  
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with fluorescence quantum yield of unity was selected as the fluorophore in this study. It is important to note that carbon acts as a quencher for the fluorescence of organic dyes.<sup>7</sup> To ensure a high fluorescence quantum yield of the prepared carbon particles,  
50 a spacer that separates dye molecules from the carbon surface is required. The quenching is most likely through Förster resonance energy transfer process.<sup>8</sup> By increasing the distance between the fluorophore and carbon surface, the quenching efficiency should be significantly decreased.<sup>9</sup> In the present study, a long alkyl  
55 chain (22-atom) was inserted between BODIPY and carbon particle surface.

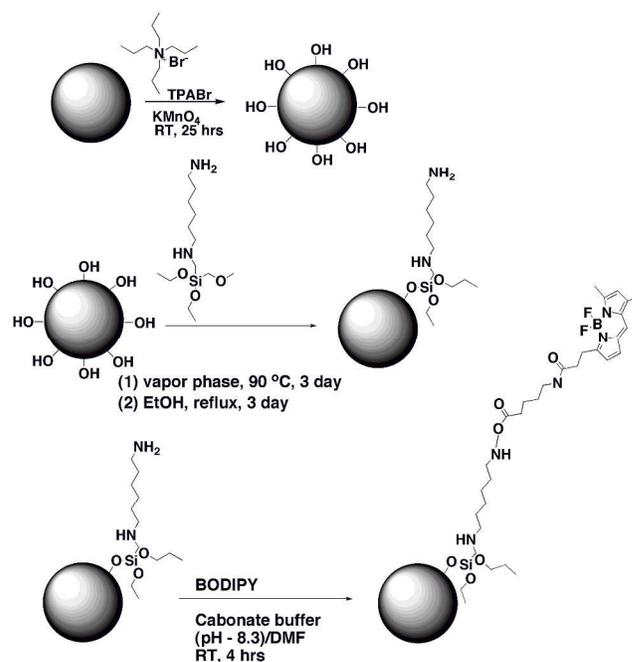
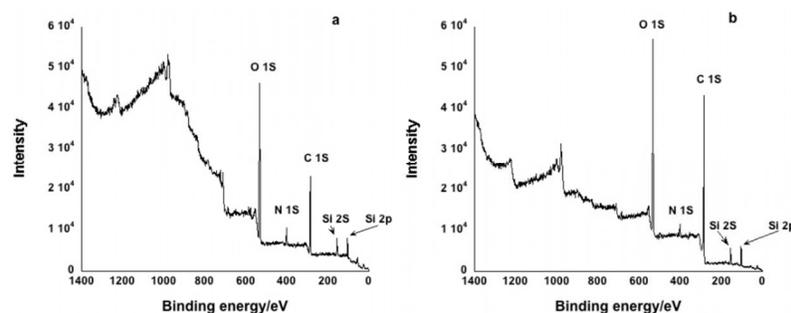


Fig.1. Synthetic scheme of fluorescent carbon.

The scheme summarizing the preparation process is shown in  
60 Figure 1. First, the carbon particles were pretreated with potassium permanganate for 24 hours at room temperature in order to generate hydroxyl groups on the surface.<sup>10</sup> The hydroxylated particles were then reacted with N-(6-aminohexyl)aminomethyl triethoxysilane (AHAMTES).

Attempting to conduct this reaction by mixing the two reagents in dry toluene under nitrogen atmosphere<sup>10</sup> results in agglomeration

due to the polymerization of the silane-coupling reagent, AHAMTES.



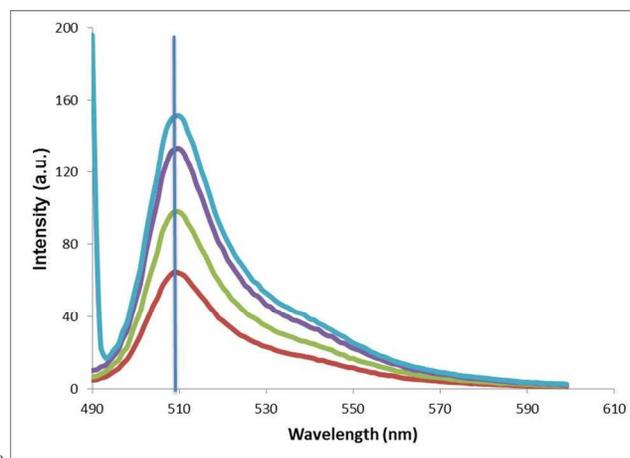
**Fig.2.** The XPS spectra of the silanized carbon particles using (a) vapor phase reaction and (b) solution phase reaction.

In order to suppress the undesired polymerization process, we have applied two distinctive methods. First, the reaction was conducted in the vapor phase.<sup>11</sup> Briefly, AHAMTES was placed in a larger flask and the carbon particles were placed in a smaller container that was suited in the larger flask. The diagram is illustrated in Figure S-1. The contact between the liquid silane-coupling reagent and carbon particles was avoided. AHAMTES vapor was generated by heating the reaction system. The vapor was transferred by diffusion and then reacted with the pre-treated carbon particles. Under this condition, the polymerization of the silane-coupling reagent on the carbon particles was effectively avoided because the polymerized products cannot vaporize due to their high molecular weight. Alternatively, the reaction was conducted in the solution phase using ethanol as the solvent. The reaction mixture was refluxed for 3 days.<sup>12</sup> Alcohols have been reported to prevent the silane-coupling reagent from polymerization<sup>11</sup> because side product of the polymerization reaction is ethanol. Therefore, in the presence of large amounts of ethanol, the polymerization reaction was inhibited. Both methods have successfully led to the preparation of carbon particles without agglomeration. Note that all the fluorescent carbons were prepared under the solution phase condition, because this reaction is less sensitive to the water concentration in the system. The surface attachment of AHAMTES was confirmed by the XPS analysis. Two representative XPS spectra from silanized 5  $\mu\text{m}$  diameter porous graphitic carbon particles (Hypercarb®) are shown in Figure 2. Clearly, the signals of Si and N that are from AHAMTES are observed.

To attach BODIPY, the functionalized carbon particles were suspended in a carbonate buffer solution containing 4, 4-difluoro-5, 7-dimethyl-4-bora-3a, 4a-diaza-*s*-indacene by vigorous shaking. The reaction was performed avoiding light by wrapping with aluminium foil in order to protect BODIPY dye from photobleaching. The particles were used without further purification.

The BODIPY modified Hypercarb and activated carbon particles exhibit fluorescent at room temperature in water. Both particles show emission at 510 nm, close to the reported emission spectrum of the BODIPY in aqueous solution.<sup>13</sup> The emission spectra of the BODIPY modified activated carbon particles at different excitation wavelengths are shown in Figure 3. The result clearly indicates that the emission of carbon particles is from the BODIPY fluorophore.

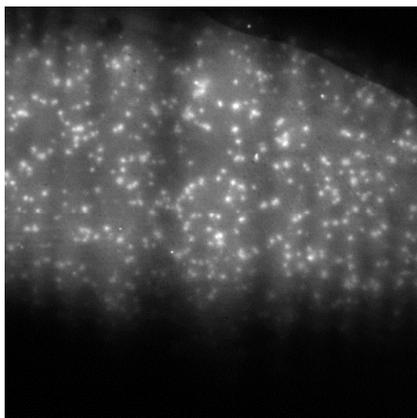
It has been commonly observed that the emission of fluorescent carbon dots is excitation-wavelength dependent due to either broad size distribution or the existence of different emissive states.<sup>14</sup> This has caused severe problems when these fluorescent carbon dots are used as sensors or fluorophore in various applications. Figure 3 shows that the emission spectra of the BODIPY modified carbon particles excited at different excitation wavelengths. The emission wavelength is clearly independent of the excitation wavelengths.



**Fig. 3.** Fluorescence spectra of BODIPY modified activated carbon using different excitation wavelengths: — 460 nm, — 470 nm, — 480 nm, — 485 nm.

The fluorescence quantum yields ( $Q$ ) of the BODIPY modified Hypercarb and activated carbon was determined by using rhodamine B in water as a reference. Dilute samples of both carbon particles in aqueous solution were prepared. Figure S-2 shows the linear dependence of the emission intensity on the absorbance indicating that our emission quantum yield measurements are valid.  $Q$  of BODIPY modified Hypercarb carbon particles and activated carbon is 0.26% and 0.48%, respectively. The fluorescence quantum yield of BODIPY modified carbon particles is lower than that of the BODIPY fluorophore in solutions, because (1) the quantum yield is determined by the total absorption, however carbon strongly extinguishing the excitation does not contribute to the emission due to the low energy transfer efficiency between the carbon particles and fluorophores.<sup>15</sup> (2) the aliphatic chain can bend over in the presence of solution, which decreases the distance between

carbon particle surface and BODIPY. As a result, the fluorescence of BODIPY may be quenched by carbon to some extent. (3) Fluorescence quenching of BODIPY by adjacent carbon particles in solution may be possible. Especially the unreacted carbon particles can interact with BODIPY and decrease the fluorescence quantum yield. The difference of the fluorescence quantum yield of the Hypercarb and activated carbon particles may be due to the inaccurate absorption measurement caused by the scattering from the carbon particles.



**Fig. 4.** An image (exposure time 0.1 s) of Hypercarb particles (with nominal diameter  $\sim 5 \mu\text{m}$ ) suspended in 1 mmol/L sodium tetraborate ( $\text{Na}_2\text{B}_4\text{O}_7$ ) solution and illuminated by blue light at a wavelength of 488 nm. The field of view is  $\sim 200 \mu\text{m}$  square.

Despite the relatively low quantum yield of the BODIPY modified carbon particles, we have successfully demonstrated the feasibility of using the fluorescent carbons as the tracers for imaging in flow velocimetry near the surface of a microchannel. A digital image of the fluorescent carbon particles prepared from Hypercarb is shown in Figure 4. Although these particles are less bright than commercially available BODIPY-labeled polystyrene (PS) particles, they are nevertheless the first, to our knowledge, conducting particles that can be used as flow tracers in microfluidic applications. Given that dielectric PS and colloidal silica particles do not (completely) follow the flow in the presence of steady (dc) electric fields,<sup>16</sup> these particles could be useful as an alternative flow tracer in electroosmotic flows driven by such steady electric fields.

## Conclusions

The water insoluble fluorescent carbon particles were prepared by chemically attaching a BODIPY fluorophore to the carbon surface. An aliphatic spacer of 22-atom was placed in between to avoid the fluorescence quenching from carbon. Compared to other fluorescent carbon, the particles prepared by this method exhibit low solubility in water. Additionally, the fluorescent carbon particles with a variety of sizes can be easily prepared. Moreover, we have successfully demonstrated the potential application of the as prepared fluorescent carbon in visualization of the movement in nanofluidic devices.

## Notes and references

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