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# Interaction between fluorescein isothiocyanate and carbon dots: Inner filter effect and fluorescence resonance energy transfer

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ABSTRACT

Carbon dots (CDs) have been widely used for the preparation of multifunctional probes by conjugation with organic fluorescent dyes. However, the effect of organic fluorescent dyes on CDs still remains poorly understood. Herein, the effect of fluorescein isothiocyanate (FITC) on CDs was explored by spectroscopic techniques at pH 5.1, 7.0 and 9.0. The fluorescent intensity of CDs was found to be quenched gradually after mixing directly with different concentrations of FITC, but the fluorescent lifetime of CDs remained unchanged. According to the results of UV–vis absorption spectra and fluorescent lifetime measurements, a pH-dependent inner filter effect (IFE) between CDs and FITC was proposed. However, the fluorescent lifetime of CDs deceased after their conjugation with FITC, implying the fluorescence resonance energy transfer (FRET) between CDs and FITC. This study has revealed two different effects of FITC on CDs with varying pH values and provided useful theoretical guidelines for further research on the interaction between other nanoparticles and fluorophores.



## 1. Introduction

Over the past several decades, the interaction between nanoparticles and fluorophores or other chemicals has attracted widespread attention among researchers, which has facilitated the development of various fluorescence nanosensors. For example, Wu et al. [1] have designed a dual-emission ratiometric fluorescent probe for detection of $Zn^{2+}$ based on fluorescence resonance energy transfer (FRET) between different-colored quantum dots and meso-tetra(4-sulfonatophenyl)porphine dihydrochloride (TSPP). Yan et al. [2] have developed a sensitive ratiometric fluorescent sensor for detection of organic phosphorus pesticides based on inner filter effect (IFE) between gold nanoparticles and quantum dots. Xu et al. [3] have established a new gold nanoparticles-based sensor for detection of thiols due to FRET between gold nanoparticles and BODIPY. These reports suggest the importance and necessity of the research on the interaction between nanoparticles and fluorophores. However, most current studies are mainly focused on quantum dots [4–7], metal nanoparticles [8–10], metal nanoclusters [11–13], and organic fluorescent dyes [14–16].

Carbon dots (CDs), a new type of fluorescent nanoparticles, have aroused strong interest among researchers due to their unique characteristics and distinct optical properties [17–21], such as good water solubility, satisfactory environmental friendliness, favorable photostability, facile surface functionalization, high biocompatibility and excellent cell membrane permeability. These attractive features indicate the prominent advantages of CDs in chemical sensing [22–25], biosensing [2,26,27], bioimaging [28–31], nanomedicine [32], and catalysis [33,34]. Recently, a series of CDs-based systems have been constructed, especially the pH sensors based on CDs and fluorescent dyes that are designed to accurately quantify the pH values in cells and even in organelles [35,36]. Generally, in the process of designing a pH sensor, the most commonly used fluorescent dye is FITC [37–41], which can be easily conjugated with a variety of nanoparticles through amino group, and the nanoparticles can still maintain their optical properties and quantum effect after conjugation with FITC.

To date, the studies on CDs and organic fluorescent dyes have mainly focused on their applications in chemical and biological fields, and little information is available on the interactions between CDs and organic fluorescent dyes such as FITC. To our best knowledge, the effect of FITC on CDs has not been studied systematically, indicating the necessity of the research on the effect of organic fluorescent dyes such as FITC on CDs.

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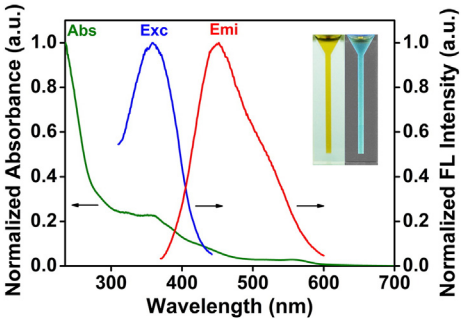


**Fig. 1.** The UV–vis absorption, fluorescence excitation and emission spectra of the as-prepared CDs at the concentration of 76.6 μg/mL. Inset: images of CDs under irradiation of sunlight (left) and UV (right, 365 nm) light.

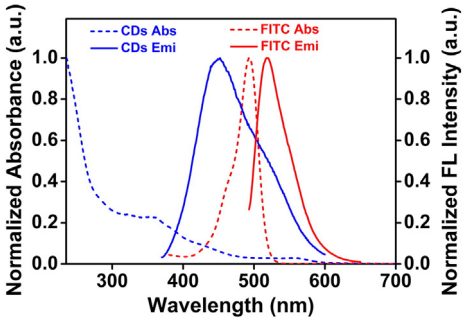


**Fig. 3.** The normalized fluorescence and UV–vis absorption spectra of CDs and FITC.

Herein, the effect of FITC on CDs was studied systematically. Due to the high sensitivity of FITC to pH, the experiment was conducted in different pH values, and the effect of FITC on CDs was explored in two different ways: mixing and conjugation.

## 2. Materials and methods

### 2.1. Apparatus

Fluorescence measurements were performed on a RF-5301PC spectrofluorometer (Shimadzu, Japan) equipped with a 20 kW xenon discharge lamp as a light source. The UV–vis absorption spectra were obtained on a UV-2450 spectrophotometer (Shimadzu, Japan) with a 1.0 cm path-length quartz cuvette. Fluorescence lifetime measurements were recorded on an FLS920 spectrometer from Edinburgh Instruments Ltd. Fourier-transform infrared (FT-IR) spectra were collected on a Nicolet Avatar-330 spectrometer (Thermo, USA) using the KBr pellet technique. Zeta potential and hydrodynamic diameters were measured with a Zetasizer Nano ZS90 DLS system (Malvern, England). Transmission electron microscopy (TEM) images were acquired by a JEM-2100F transmission electron microscope (JEOL, Japan) operating at an acceleration voltage of 200 kV. A PHS-3C pH-meter (INESA, China) was used for pH measurements.

### 2.2. Reagents

4,7,10-Trioxa-1,13-tridecanediamine (TTDDA, 96%) and fluorescein isothiocyanate (FITC, 95%) were purchased from Tokyo Chemical Industry Co. Ltd. Citric acid anhydrous (99.5%) and quinine sulfate dihydrate (99.0%) were obtained from Aladdin Regent Co. Ltd. Glycerin, ninhydrin and other common solvents or salts were obtained from Sinopharm Chemical Regent Co. Ltd. Ultrapure water with a resistivity of 18.2 MΩ·cm was used throughout the experiments. All these reagents were of analytical grade or better and used as obtained.

### 2.3. Synthesis of amino-functionalized carbon dots (CDs)

The amino-functionalized CDs used in this study were synthesized as previously reported [35]. Briefly, 15.00 mL glycerin was mixed with 1.00 mL TTDDA and heated to 220 °C under nitrogen atmosphere. Subsequently, 1.0054 g citric acid anhydrous was added and the temperature was kept at 220 °C for 3 h. The obtained dark brown turbid mixture was purified via exhaustive dialysis to remove the impurities and centrifuged to remove the precipitate. The supernatant was collected and stored in dark at 4 °C for further use. The concentration of the as-prepared CDs solution was determined to be 3.83 mg/mL.

### 2.4. Preparation of CDs-FITC conjugates

CDs-FITC conjugates were prepared using a method analogous to others [35]. Briefly, 5.00 mL of the amino-functionalized CDs solution prepared above (3.83 mg/mL) was mixed with 15.00 mL of FITC (177 μM in water) in 0.10 M $NaHCO_3$ (pH = 8.31). After reaction at room temperature for 36 h, the resulting labeled CDs solution was purified via thorough dialysis using a dialysis membrane with a molecular weight cut-off of 1000 Da against 0.10 M $NaHCO_3$ for 24 h and ultrapure water for another 24 h (48 h in total) to remove the unbound FITC. The conjugates were then stored at 4 °C as the stock solution for subsequent spectral experiments.

### 2.5. Investigation of the pH-dependent effect of FITC on CDs

In a typical experiment to test the pH-dependent effect of FITC on CDs, different volumes of 177 μM FITC in water were added to 0.31 mg/mL CDs solutions in 20 mM phosphate buffer solution in the

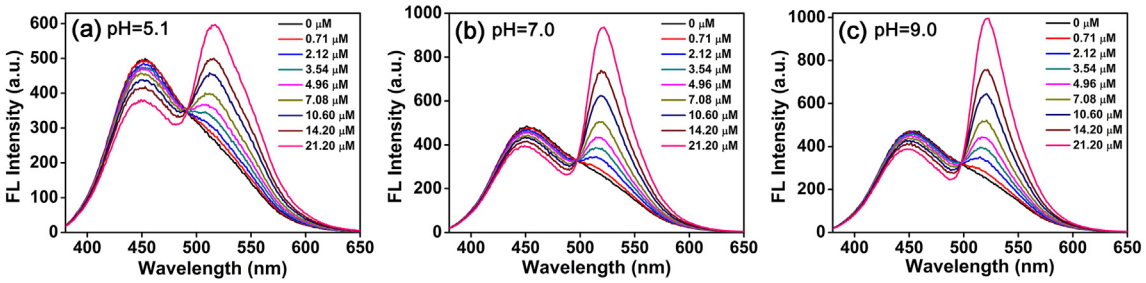


**Fig. 2.** Effect of FITC on the fluorescence spectra of CDs in phosphate buffer solution with different pH values: (a) 5.1, (b) 7.0 and (c) 9.0. The concentration of CDs was fixed at 0.31 mg/mL, and the concentrations of FITC were 0, 0.71, 2.12, 3.54, 4.96, 7.08, 10.60, 14.20 and 21.20 μM separately.