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INNER FILTER EFFECT: AN ISSUE COULD LEAD TO MISINTERPRETATION OF FLUORESCENCE MEASUREMENTS OF CARBON DOTS

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ABSTRACT

This paper aims to provide a notification related to the inner filter effect (IFE) that can affect the accuracy of measurements in fluorescent spectroscopy. This effect can cause the measured fluorescence to be distorted than it should be, leading to inaccurate interpretation of experimental results. Especially, this can become more pronounced when the substances are highly concentrated. Here, carbon dots (CDs) were experimentally employed as an illustration to this effect when measuring photoluminescence (PL). Contour plots of the PL emission were employed to visualize spectral changes under different optical densities and measurement configurations. A pronounced discrepancy was observed between the high- and low-concentration samples, demonstrating the role of IFE in fluorescence results. These findings serve as a cautionary reference for researchers working with fluorescent nanomaterials, emphasizing the need to control experimental parameters and validate data interpretation, particularly in PL studies of CDs.

Keywords: carbon dots, fluorescence, inner filter effect, nanomaterials

1. INTRODUCTION

Nowadays, well-developed instrumental devices allow us to observe the natural world in more detail. There are many ways to probe the characterisation of the objects based on the expected application. In the optical approach, the objects' behaviors are considered through the interpretation of the effects associated with light [1]. During the last two decades, a great deal of attention has been focused on the structures significantly smaller than the wavelength, and many essential features occur in this region, which is called nanostructured materials [2].

From the optical point of view, photoluminescence is a basic property and an important step in the characterization of the optical characteristics of nanomaterials. Although the procedure appears simple and straightforward with the availability of commercial spectrometers, there are important and often underestimated sources of error [3]. Especially if one has no information about the concentration of a fluorophore, it could lead to an undesirable effect in fluorescence measurements, called the inner filter effect [3-5]. This effect can cause the measured fluorescence to be distorted more than it should be, leading to inaccurate interpretations of experimental results. Especially, this can become more pronounced as the concentration of measured substances increases [5-7].

This work aims to demonstrate an inner filter effect based on carbon dots (CDs) derived from waste wine cork, which is considered a new generation of fluorescent quantum dots without toxic heavy metals. To do that, we use the UV-Vis and fluorescence spectrometers to study the spectral data of CDs. Two samples at high and low concentrations were prepared to investigate. The excitation emission map was used to quickly visualize fluorescent patterns and should point out an important factor that affects the fluorescence emission spectral profiles, which is the inner filter effect (IFE). We will discuss whether this contribution is dominant or not based on the relationship between the fluorescence intensity and the absorbance of the obtained CDs at low concentrations.

2. EXPERIMENT

For sample preparation, we follow the same process as in the previous publication [8]. Namely, we utilized discarded wine corks as starting materials. These corks (5.0 g) underwent a preliminary cleaning process with water to eliminate any surface impurities and were subsequently dried in an oven. Subsequently, the corks were cut into small pieces and mixed with 80 mL of distilled water in a 100 mL Teflon-lined autoclave. The resulting mixture was subjected to a temperature of 220°C for a duration of 4 hours in an oven. After naturally cooling to room temperature, the resulting solution took on a brown-black carbonized appearance and was subjected to a rough purification process using a 0.22 μm microporous membrane. Following this, the solution was centrifuged at 14000 rpm for 15 minutes to eliminate larger particles. Finally, the produced CDs were stored at 4°C for future investigation.

We conducted a comprehensive analysis of the structural characteristics of the obtained CDs using various analytical techniques. X-ray diffraction (XRD) was used to assess the crystallographic properties (D8 Advance instrument from Bruker, Germany). We examined the size and morphology of the CDs using a JEOL JEM-1010 TEM equipped with an 80 kV accelerating voltage (JEOL, Japan). To investigate the optical properties, we recorded UV-Vis absorption spectra on a GENESYS 10S UV-Vis spectrophotometer (Thermo Scientific, USA). Finally, for fluorescence analysis, we utilized an FS5 spectrofluorometer (Edinburgh Instrument, UK).

3. RESULTS AND DISCUSSION

The phase characteristics of the obtained CDs were elucidated through X-ray diffraction. The XRD pattern displayed in Fig.1A revealed a broad peak at 2θ~22°, indicative of the (002) lattice spacing, characteristic of highly amorphous carbon-based materials [8]. The image provided in Fig.1B indicates that transmission electron microscopy (TEM) clearly demonstrates the obtained particles are uniformly dispersed without evident aggregation.

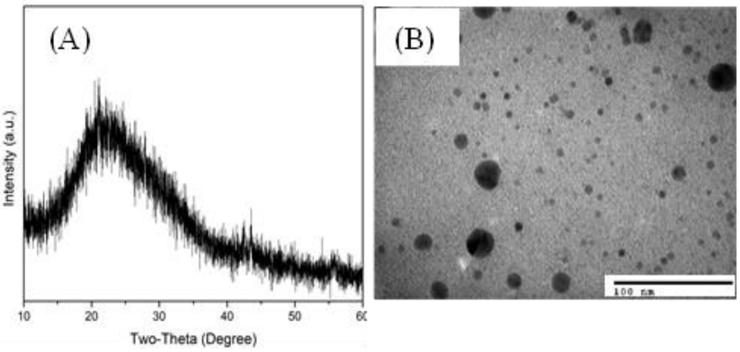

Fig. 1. (A) XRD pattern and (B) TEM image of the obtained CDs with the scale bar of 100 nm

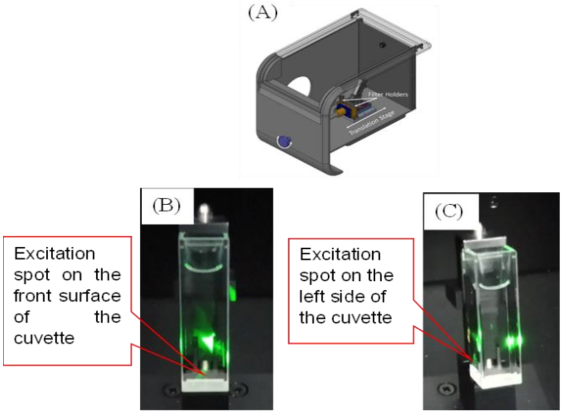

Fig. 2. (A) Representation of SC-10 front-face sample holder [9]. Position of the excitation spot on the front surface (B) and left side (C) of the cuvette.

Next, to experimentally clarify the role of IFE in fluorescent measurement, the SC-10 front-face sample holder of the FS5 spectrofluorometer (Fig. 2A) was utilized [9]. Together, the excitation spot was irradiated on the front surface and left side of the cuvette (for comparison) to detect a fluorescent signal [3]. Fig. 2B and C are the photographs of spot light on the cuvette. To avoid the IFE, as recommended, the measured sample has an optical density below 0.1 over the excitation wavelength ranging from 340 nm to 500 nm [3, 5]. As a control, the signal of a sample with a high concentration (optical density above 0.1) was also detected.

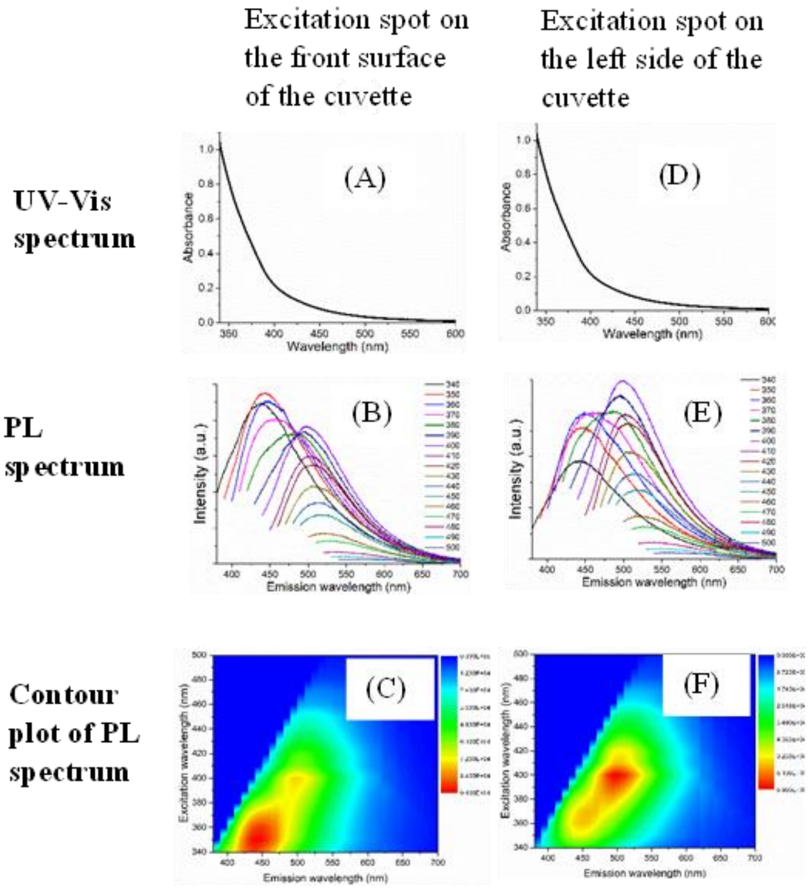

Fig. 3. (A, D) The UV-Vis spectrum of high OD samples. (B, C, D, E) The PL and contour plot of the PL spectrum of high concentration sample whereby the excitation spot was irradiated on the front surface and left side of the cuvette.

To further clarify the experimental configuration, the measurement setup was compared with the standard cuvette geometries described in the literature [5]. The front-face configuration employed in this study is equivalent to the reflection-type geometry [5], in which both excitation and emission beams interact with the front surface of the sample. This geometry is known to minimize the inner filter effect (IFE) by significantly reducing the effective pathlength for both excitation and emission light, thus enhancing the accuracy of the fluorescence signal, especially in optically dense media. In contrast, the left-side configuration in our experiment corresponds to the transmission-type geometry [5], where the emission is detected from the side opposite to the excitation, resulting in a longer light path through the sample and higher sensitivity to IFE.

According to Panigrahi and Mishra [5], significant IFE leads to measurable changes in fluorescence intensity and spectral shape depending on the detection geometry. In our results presented below, the fluorescence spectra recorded from low-concentration samples under both front-face and left-side configurations were nearly identical, indicating that IFE was negligible under these conditions. This observation validates the theoretical prediction that IFE becomes significant only when the optical density exceeds a certain threshold.

It should be noted that due to instrumental constraints, the present study was limited to the front-face (reflection-type) and left-side (transmission-type) detection geometries. Other configurations, such as right-angle or total internal reflection, which are known to further reduce IFE, could not be implemented with the current FS5 spectrofluorometer setup.

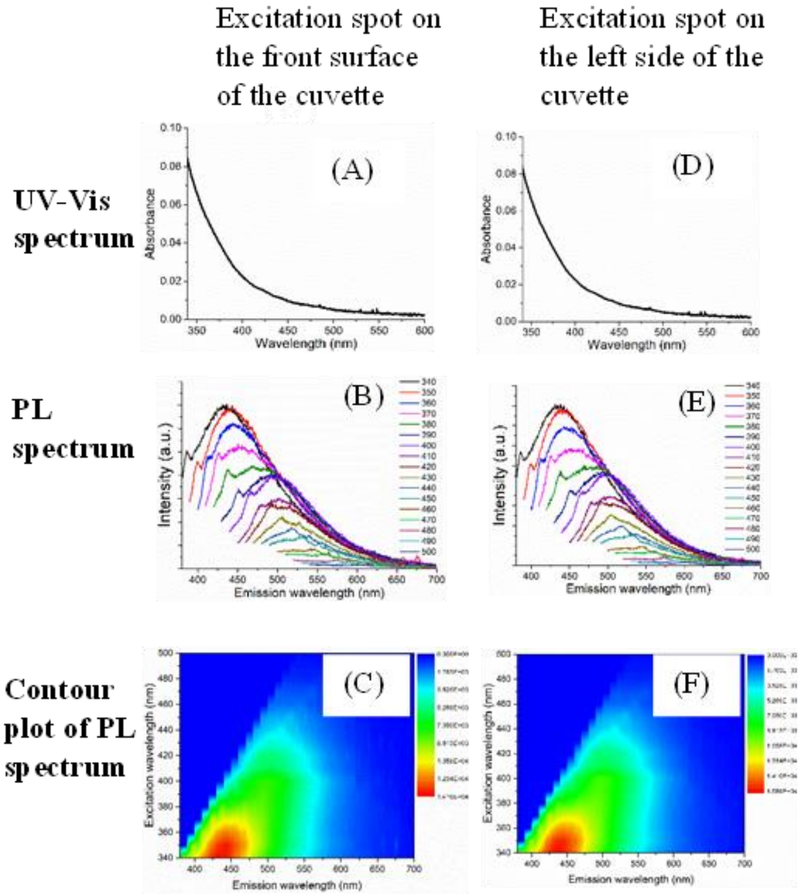

Fig. 4. (A, D)The UV-Vis spectrum of low OD samples. (B, C, D, E) The PL and contour plot of the PL spectrum of low concentration sample whereby the excitation spot was irradiated on the front surface and left side of the cuvette.

The absorption and emission spectra of the obtained CDs with high and low optical density (OD) are shown in Figs. 3 and 4. Here, photoluminescence (PL) emission spectra of all solutions were recorded at an excitation wavelength from 340 to 500 nm (in 10 nm increments starting from 340 nm). The OD values of high- and low-concentration samples at the wavelength of 340 nm are 1.037 (Figs. 3A and D) and 0.084 (Figs. 4A and D), respectively. For the sample at high concentration, the PL (Figs. 3B and E) and contour plot (Figs. 3C and F) of the PL spectrum show a strong discrepancy in fluorescence emission spectral profiles in two cases of illumination. In contradiction, as shown in Fig. 4, we found that the PL spectra of the low-concentration sample were almost similar when the excitation beam was illuminated on the front surface or left side of the cuvette.

From a quantitative perspective, the observed fluorescence intensity ($F_{observed}$) is attenuated by both primary and secondary inner filter effects [3]. According to Lambert-Beer's law, these attenuations are due to absorption at the excitation and emission wavelengths. The corrected fluorescence intensity ($F_{corrected}$) can be approximately calculated using the following expression:

$$F_{corrected} = F_{observed} . 10^{\frac{(A_{ex}+A_{em})}{2}} \tag{1}$$

where A_{ex} is the absorbance of the sample at the excitation wavelength and A_{em} is the absorbance at the emission wavelength.

This formula, first proposed by Lakowicz [3], assumes a symmetric fluorescence observation field at the center of a 1 cm cuvette, and is valid for optically dilute solutions (typically OD < 0.1). In more complex or concentrated systems, more advanced models like the Parker-Holland method or empirical calibration are needed to fully correct for IFE [5].

To provide a quantitative demonstration, we extracted the experimental data of A_{ex} (340 nm), A_{em} (435 nm) and $F_{observed}$ for two samples at high and low concentrations, as listed in Table 1. Based on these values, the corrected fluorescence intensity $F_{corrected}$ was calculated and illustrated in Fig. 5. At high concentration (OD = 1.0369 at 340 nm), the fluorescence intensity requires a substantial correction factor of ~3.73, reflecting significant attenuation due to primary and secondary IFE. In contrast, at low concentration (OD = 0.0836), the correction factor is minimal (~1.116), indicating negligible IFE. This stark contrast in correction factors between the two concentrations robustly confirms the presence of IFE in CDs, validating that measurements at these two concentrations are sufficient to establish its impact on fluorescence spectroscopy.

Table 1. *Experimental values of* A_{ex}, A_{em} *and* $F_{observed}$ *for CDs solutions at high and low concentrations under two different measurement configurations.*

Sample	**Configuration**	A_{ex} **(340nm)**	A_{em} **(435nm)**	$F_{observed}$ (a.u)	$F_{corrected}$ (a.u)
Low concentration	Front surface	0.0836	0.0123	13500	15066
Low concentration	Left side	0.0836	0.0123	12000	13392
High concentration	Front surface	1.0369	0.1074	6000	22380
High concentration	Left side	1.0369	0.1074	4000	14920

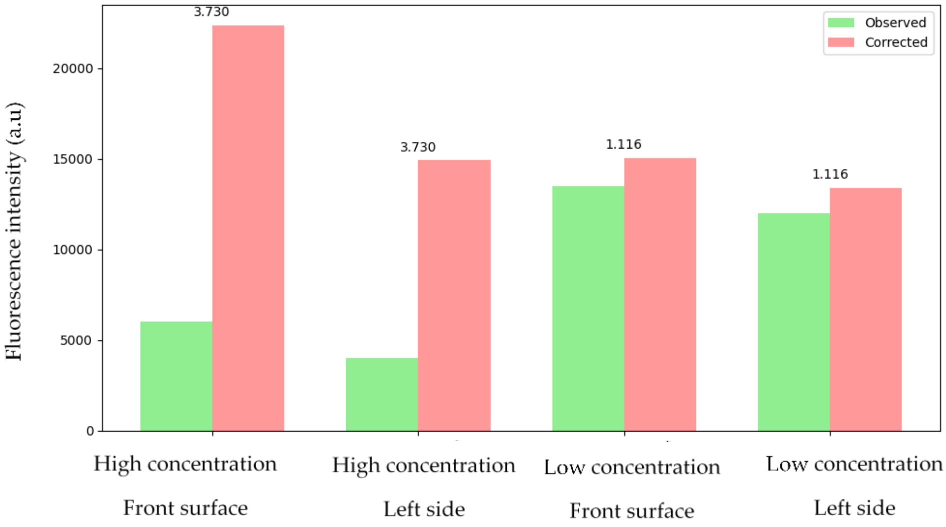

Fig. 5. Observed and corrected fluorescence intensity of CDs

We should also further consider the obtained spectra shown in Fig. 3, in which this could lead to a misinterpretation of the fluorescence mechanism of CDs. In early studies, CDs normally exhibit the dependence of the emission on the excitation wavelength [10, 11]. For CDs, the PL mechanism is the most important issue in terms of investigation [12]. Although the origin of fluorescence in CNDs is not yet entirely understood, there is confirmation that two emission centers are similar to the data shown in Fig. 4F [13, 14]. In a previous publication, Pyng Yu et al. showed evidence of dual fluorescence bands when they investigated the temperature-dependent spectroscopic results of CDs [13]. The shorter emission band was attributed to the core state emission, whereas the longer one resulted from the surface state emission. More recently, it has been found that fluorescent CDs synthesized from tofu wastewater and chitosan also present this effect [14, 15]. Similarly, in our case, there is an obvious shift as the excitation wavelength varies, as shown in Figs. 3B and E. Particularly, the corresponding contour of PL spectra shown in Fig. 3F revealed two fluorescence bands, with the emission intensity achieving the strongest value in the longer excitation wavelength region. At first glance, the data shown in Fig. 3F is surprising since the core and surface of CDs play an important role with respect to the PL spectrum, but it certainly cannot be correct. The erroneous result in Fig. 3 is a consequence of incorrect treatment of sample concentration, which leads to the predominant emergent IFE effect and generates distorted PL shapes during fluorescence measurement. For a more detailed interpretation of the fluorescence behavior of the obtained CDs, readers are encouraged to refer to our previous publication [16].

(A)

Absorbance	Fluorescence intensity
0.084	1.27888242E+4
0.068	8.65270410E+3
0.061	7.24238770E+3
0.045	5.49401074E+3
0.040	4.88736719E+3
0.036	3.64297070E+3

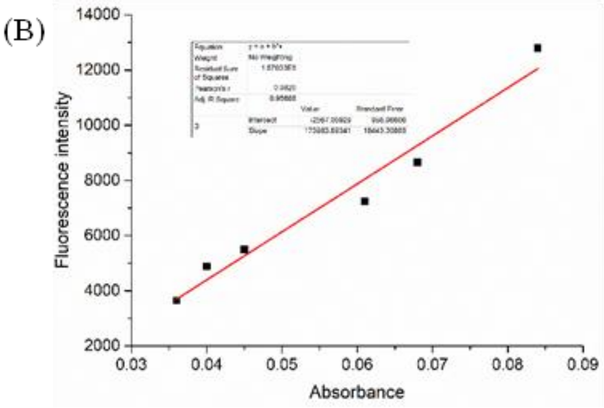

Fig. 6. (A) Data from absorption measurements and fluorescence spectra. (B) Absorbance vs. fluorescence intensities. λ_{ex}=340 nm, λ_{em}=435 nm.

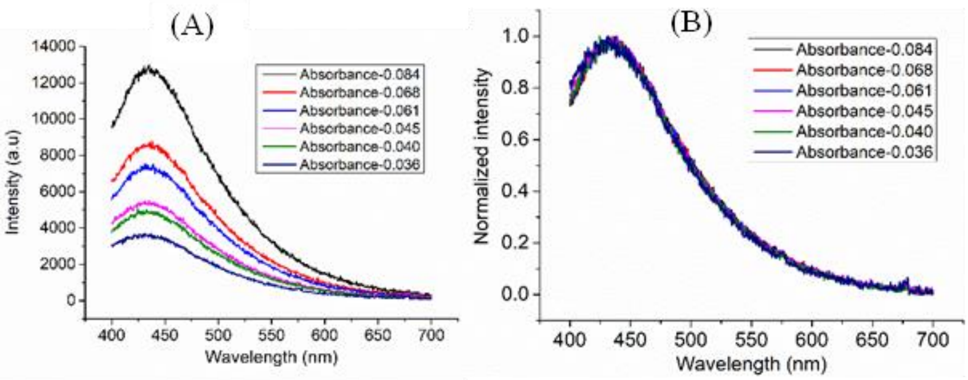

Fig. 7. (A) Emission spectra of the obtained CDs at different concentrations and (B) the corresponding normalized spectra. λ_{ex}=340 nm.

Based on the obtained data shown in Fig. 6, we finally considered the emission intensity and spectral position of the peak maximum at different concentrations. Here, the incident beam was illuminated on the front surface of the cuvette. Namely, samples were excited at a wavelength of 340 nm, and the PL curve ranges in wavelength from

400 to 700 nm. The graph of the absorbance (at 340 nm) vs. fluorescence intensities (at 435 nm) obtained from the experiment and the resulting linear fits (R2 = 0.9) are shown in Fig. 5. Together, the emission spectra of the obtained CDs at different concentrations and the corresponding normalized spectra are shown in Fig. 7. As pointed out in Fig. 7B, the positions of the peaks are almost coincident as concentration changes. These results further imply that IFE was not present [3].

4. CONCLUSIONS

In this study, the influence of the inner filter effect on the spectral profiles of fluorescence emissions was investigated. We experimentally show that IFE could induce substantial alterations in the overall shape of spectra, leading to shifts in the positions of peak. Two simple tricks were presented in which the spot light of the exciation source and the relation between absorbance and fluorescence intensities should be considered before taking the measurement. Consequently, when utilizing a conventional spectrofluorometer to measure fluorescence, the recorded emission may not faithfully represent the true fluorescence characteristics of the sample if we do not consider the concentrations of fluorophores. These distortions in fluorescence spectra compromise the accuracy of measurements and potentially lead to misinterpretations of the emission properties of nanomaterials, as pointed out in the case of CDs.

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LỌC CỤC BỘ: HIỆU ỨNG DẪN ĐẾN SUY LUẬN SAI KẾT QUẢ ĐO PHỔ QUANG PHÁT QUANG CỦA HẠT CARBON NANO

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TÓM TẮT

Bài báo này trình bày kết quả thực nghiệm liên quan đến hiệu ứng lọc cục bộ (Inner Filter Effect - IFE) có thể ảnh hưởng đến độ chính xác của các phép đo trong quang phổ huỳnh quang. IFE có thể dẫn đến sự sai lệch kết quả đo phổ huỳnh quang, dẫn đến việc diễn giải không chính xác các kết quả thí nghiệm. Đặc biệt, hiệu ứng này trở nên rõ rệt hơn khi nồng độ của các chất được đo ở mức cao. Trong nghiên cứu này, chấm carbon đã được sử dụng làm mẫu đo thực nghiệm để minh họa cho hiệu ứng IFE khi đo quang phát quang (PL). Hai mẫu với nồng độ cao và thấp đã được đối chiếu với nhau, và biểu đồ đường đồng mức của phổ PL được sử dụng để cho phép chúng ta quan sát được vị trí các đỉnh phổ phát quang. Kết quả cho thấy sự khác biệt rõ rệt giữa hai trường hợp. Do đó, nghiên cứu này được kỳ vọng sẽ cung cấp thêm thông tin nhằm thực hiện đúng phép đo PL đối với hạt nano carbon.

Từ khóa: chấm carbon, huỳnh quang, hiệu ứng lọc cục bộ (inner filter effect), vật liệu nano.

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Lĩnh vực nghiên cứu: Vật liệu carbon nano, Cộng hưởng plasmon bề mặt, và các hoạt động thực hành STEM.

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Lĩnh vực nghiên cứu: Quang học, Khoa học vật liệu, Vật liệu nano.