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# Functionalized Carbon-based Quantum Dots: Optical Characterization and Potential Application as Bio-fluorophore

R A T Cruz<sup>1,2</sup>, A N Soriano<sup>2</sup>, P A N de Yro<sup>1,2</sup>, G M O Quiachon<sup>1</sup>, C S Emolaga<sup>1</sup>, M L M Ysulat<sup>1</sup>, U G Bigol<sup>1</sup> and B A Basilia<sup>1,2</sup>

<sup>1</sup>Industrial Technology Development Institute, Department of Science and Technology, General Santos Avenue, Bicutan, Taguig City, 1631 Philippines

<sup>2</sup>School of Chemistry and Chemical Engineering, Mapua University, Intramuros, Manila, 1002 Philippines

E-mail: [cruzrolandandrew@gmail.com](mailto:cruzrolandandrew@gmail.com)

**Abstract.** Carbon quantum dots (CQDs), in comparison to heavy metal-based quantum dots offer renewable, non-toxic, low cost and easy synthesis production route while having excellent physicochemical properties for biomedical or environmental use. This paper discusses development and application of functionalized carbon quantum dots from glycerol as primary carbon source and tetraethylene pentamine as functionalizing agent. As-synthesized CQDs were characterized by Fourier Transform Infrared Spectrometer (FTIR), UV-Vis Spectrophotometer and Spectrofluorometer. FTIR spectra confirmed functionalization of resulting CQDs with emission wavelength peaks at 314.18 nm and 381.15 nm and observed strong blue luminescence under UV lamp. The performance of produced CQDs as bio-fluorophore for Gram-stained bacterial models was validated. Results indicated that CQDs can effect fluoresced images but a more distinct image can be observed on *S. aureus* compared to *E. coli*. An attachment mechanism of carbon quantum dots to the bacteria surfaces was also proposed here.

## 1. Introduction

The advent of the proliferation of fluorescent materials for various biomedical and environmental uses is leaning towards the adoption of naturally-based source materials. Currently, quantum dots, as these fluorescent materials are commonly called, are made up of organic dyes and heavy metals including cadmium selenide [1],[2]. Their uses in the biomedical and environmental field are restricted because of the hazard posed more than the benefits. Identification of carbon as possible alternative starting material for quantum dots is already being explored. Carbon quantum dots (CQDs) are nanosized materials measuring below 10 nanometers and are made up of quasi-spherical nanoparticles of amorphous to nanocrystalline core with hybridized carbon insertions. The CQDs apparent small size causes the variation of their properties as compared to their bulk material as described by the quantum effect mechanism. Smaller-sized CQD has higher band gap energy which means it requires higher amount of energy to excite the particle from its ground state due to stronger attractive forces. The CQD size must be small enough to effect quantum confinement thus forming surface emissive energy traps; and the addition of chemical functionalizing moieties provide stability to these energy traps that improve their properties [1]. These carbon quantum dots revolutionize the development of fluorescent



materials by offering renewable, non-toxic, low cost and easy synthesis production route while maintaining excellent physicochemical & photochemical properties for intended biomedical or environmental uses. One potent application of CQD is its use as fluorophores especially in bacteriological studies. The CQD offers possible immediate detection or labelling and imaging of microorganisms present in various systems such as food or water supply due to their fluorescence under a fluorescent microscope [3].

This paper discusses the development of carbon quantum dots from glycerol as primary carbon source and tetraethylene pentamine (TEPA) as functionalizing agent. Also, the chemical make-up and optical characteristics of produced CQDs are determined and described in this study. Validation study for the application of the resulting CQDs as fluorophores for Gram-stained bacteria and their proposed attachment mechanism are reported here.

## 2. Methodology

### 2.1. Materials and apparatus

Glycerol (98.5% purity) and Tetraethylenepentamine (TEPA) were obtained from Sigma Aldrich. Both raw material chemicals were of analytical grade and were used as received without any further treatments or purification. A Tuttnauer Model 2340E autoclave with 200 L stainless steel chambers was also used for the prepared solutions of carbon quantum dots.

### 2.2. Synthesis and functionalization of carbon quantum dots

Carbon quantum dots were synthesized from glycerol through hydrothermal carbonization followed by chemical functionalization of the produced carbon core effected by TEPA. For the CQD solutions, a 15 ml glycerol was combined with 5 ml of TEPA or 25% TEPA in glycerol and; another 17.5 ml glycerol with 2.5 ml of TEPA or 12.5% TEPA in glycerol. Both glycerol-TEPA solutions were stirred for 15 minutes using magnetic stirrer to ensure mixture uniformity. These solutions were then transferred to a Teflon-lined stainless steel autoclave under the following operating conditions: temperatures of 180°C and 220 °C and; durations of 4 hours and 12 hours. The autoclaves were then cooled to room temperature. The resulting yellowish colored viscous solutions were collected and stored in glass sample bottles for analyses and application.

### 2.3. Optical characterization of synthesized cqds and performance testing as bio-fluorophore

The presence of specific functional groups in the chemical make-up of the produced CQDs were validated by a Fourier Transform Infrared Spectroscopy (FTIR) Perkin Elmer Spectrum RX I over the wavenumber ranging from 400-4,000  $\text{cm}^{-1}$  corresponding to the shifting of functional group peak readings. The maximum absorption wavelengths which correspond to the excitation spectra for the synthesized CQDs were determined using Ultraviolet-Visible Spectrophotometer (UV-Vis) UH5300. The emission wavelength of the produced CQDs was measured by Fluorescence Spectrophotometer Hitachi F-7000 which corresponds to the fluorescence measurement of each CQD solution. The *E. coli* and *S. aureus* bacterial strains were observed under Olympus BX51 microscope with fluorescence attachment using DAPI filter cubes of 350-370 nm excitation wavelength. The application of CQD as a bio-fluorophore was studied using *Escherichia coli* and *Staphylococcus aureus* as representative Gram-negative and Gram-positive bacterial model, respectively. These bacteria were subjected to in vivo staining technique and were observed under a fluorescence microscope for the image contrast effected by CQD to the model organisms.

## 3. Results and discussion

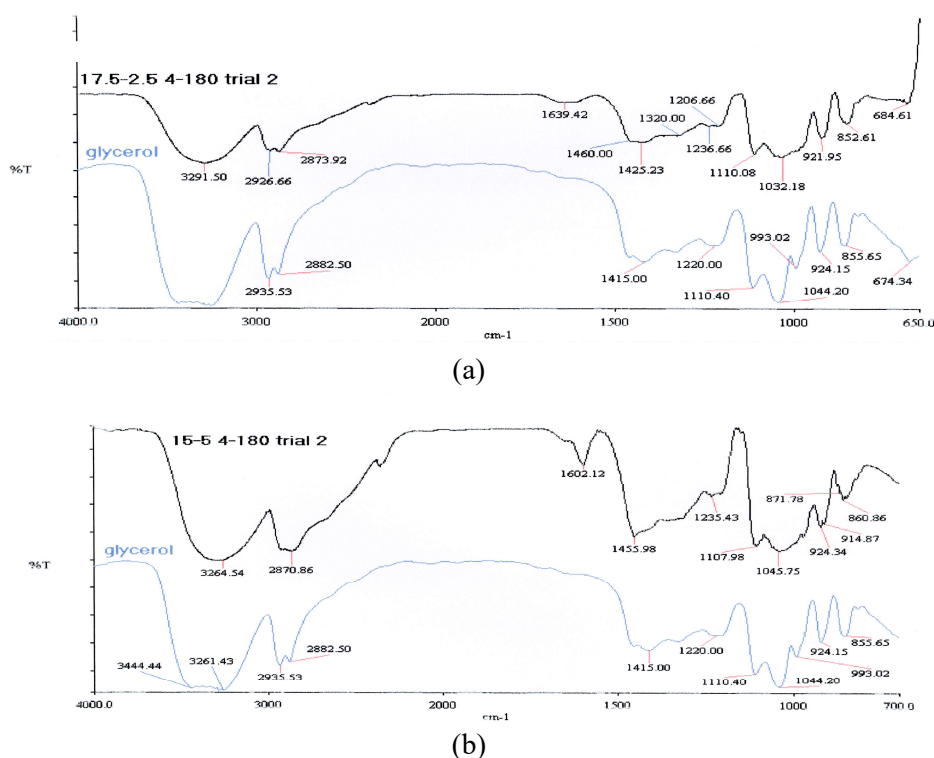
### 3.1. Characterization of CQDs

The FTIR spectra of the developed CQDs showed an observable broad band between 3000 to 3500  $\text{cm}^{-1}$ . This indicated the presence of O-H bond with peaks centering at 3291.50  $\text{cm}^{-1}$  and 3264.54  $\text{cm}^{-1}$  which also suggested existent hydrogen bonding and vibrational stretching. The bending vibration of N-H bonds is observable at 1650 to 1580  $\text{cm}^{-1}$  suggesting the conjugation of amine groups from TEPA.

Also, the presence of N-H bending in this region supports that N-H bonds exist for primary amine groups but were not observable due to weaker registers compared to broad O-H stretching which it overlaps with at 3300 to 3000  $\text{cm}^{-1}$ . In the C-H band region at 2850 to 3000  $\text{cm}^{-1}$ , C-H stretching for both developed CQDs is attributed to  $\text{sp}^3$  hybridization of the carbon atoms. The presence of  $\text{sp}^3$  hybridized C-H bonds confirms that the stability of these nanoparticles can be attributed to the formation of  $\text{sp}^3$  hybridizations in their molecular orbitals [2].

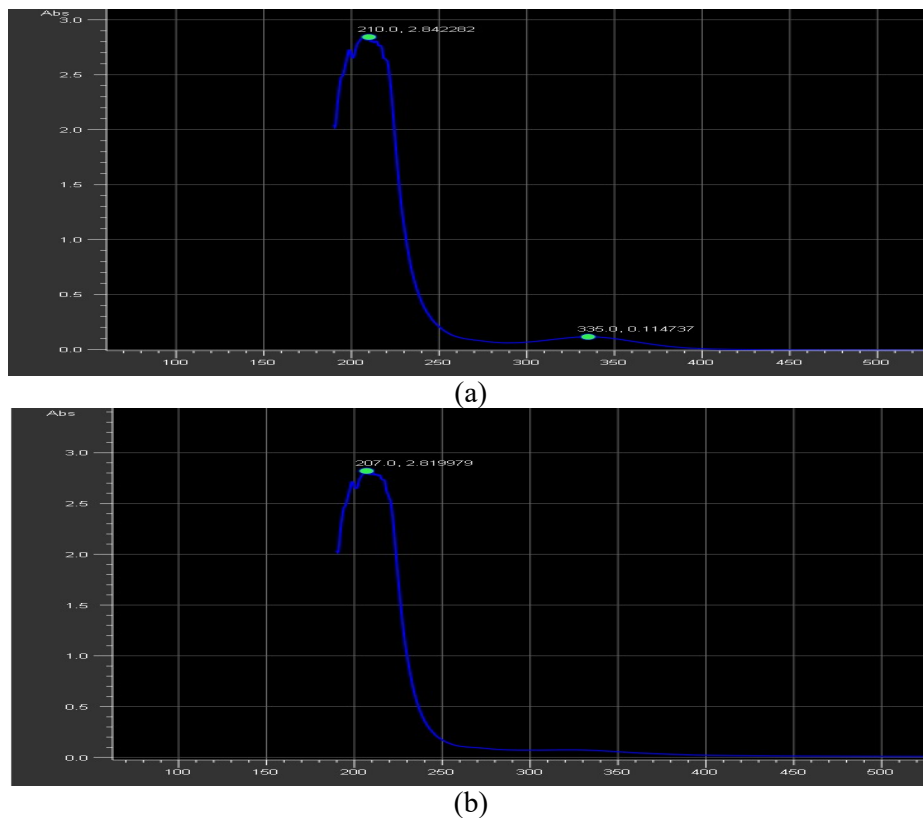
**Table 1.** FTIR spectra and corresponding functional groups

Wavelength	Functional Group
3000-3500 $\text{cm}^{-1}$	O-H stretching (Hydroxyls) N-H stretching ( $1^\circ$ Amines due to N-H bending)
2850-3000 $\text{cm}^{-1}$	$\text{sp}^3$ hybridized C-H bond
1580-1650 $\text{cm}^{-1}$	N-H bending



**Figure 1.** FTIR spectra of CQDs in the following conditions: (a) 12.5% TEPA in glycerol under 180°C for 4 hours and (b) 25% TEPA in glycerol under 180°C for 4 hours

The UV Vis spectra of developed CQDs recorded close maximum absorbance peaks of 207 and 210 nm, both wavelengths are under the UV region. This strong absorbance peak range in the UV region is suggestive of the  $\pi$ - $\pi^*$  transition of conjugated  $\pi$ -bonds of CQDs. It was observed that some CQD solutions showed more than a single absorbance peak but were still within the identified UV absorption band.

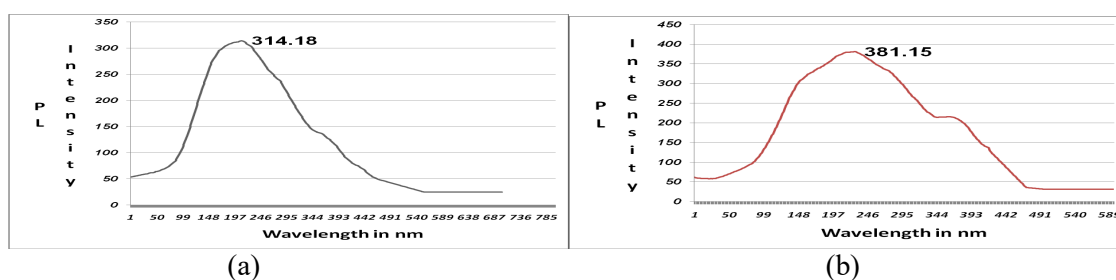


**Figure 2.** UV Vis spectra of CQD solutions prepared under the following process conditions: (a) 12.5% TEPA in glycerol under 220 °C for 4 hours and (b) 25% TEPA in glycerol under 220 °C for 12 hours



**Figure 3.** Developed CQD solutions showing strong blue luminescence under UV lamp

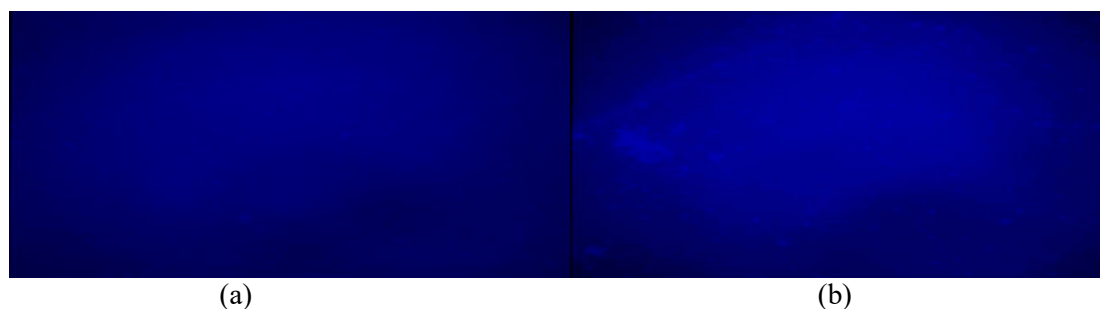
The developed CQDs were bombarded with an excitation wavelength of 210 nm taken from the maximum measured reading by the UV Vis Spectrophotometer. Maximum photoluminescence (PL) emission wavelengths peaked at 314.18 nm and 381.15 nm validating the strong blue luminescence under UV lamp with wavelength at 365 nm. The ability of CQDs to exhibit a strong blue fluorescence when placed under a UV lamp is also an indication of the formation of small-sized CQDs. Smaller CQDs have higher band gap energy which means it requires higher amount of energy to excite the particle from its ground state due to stronger attractive forces [4],[5]. This phenomenon results to shorter wavelength, higher frequency and closer luminescence to the blue end of the light spectra as observed in the developed CQDs in this study.



**Figure 4.** PL Intensity of CQDs prepared under the following process conditions: (a) 25% TEPA in glycerol under 180 °C for 4 hours and (b) 12.5% TEPA in glycerol under 180 °C for 12 hours

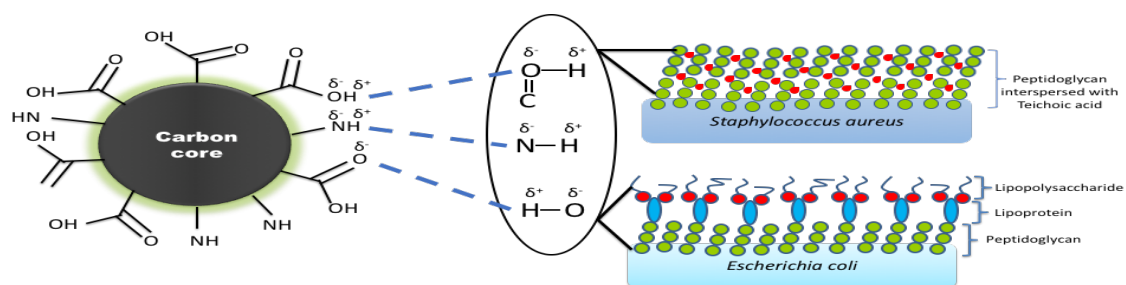
### 3.2. Application as bio-fluorophore and plausible attachment mechanism

It can be seen in the fluorescence images of the bacterial models that CQDs effected a more observable fluoresced imaging of *S. aureus* compared to *E. coli*. This phenomenon can be attributed to the surface structures present in both microorganisms which allows for the unique interaction between CQDs and the Gram-stained bacteria suggestive of a plausible attachment mechanism.



**Figure 5.** Fluorescence microscope images of representative Gram-stained bacteria with CQD solutions as imagers: (a) *Escherchia coli* (b) *Staphylococcus aureus*

Typical Gram-negative bacteria, such as *E. coli*, consist of an outer membrane made up of lipoproteins and lipopolysaccharides (i.e. phospholipids with negative polar head) over a thin, peptidoglycan wall. For Gram-positive bacteria like *S. aureus*, a singular but thicker peptidoglycan wall also exists, with observably absent phospholipid layer, interspersed with teichoic acid, which is an anionic polymer, on its surface. It was established that both Gram-stained bacteria have net negative charges based on these structures [6]. This strong blanket of negative charges enveloping *E. coli* and *S. aureus* attracts the positive particles of CQDs inducing temporary polarity that is supplemented by the formation of hydrogen bonds. A closer look to the chemical groups of the outer membranes of these bacteria show an abundance of amines (–NH) and hydroxyl (–OH) molecules common in amino- and carbohydrate-based structures. Similarly, chemical moieties of CQDs, also confirmed by the FTIR characterization, are mostly composed of hydroxyl (–OH) branching from carboxyl groups and amine (–NH) functional groups. These molecules tend to have greater negative charge density towards the nitrogen and oxygen ends due to high electronegativity and corresponding lone pairs. This behavior leaves the hydrogen atom with a positive charge density. The localization of positive charge in hydrogen atoms and deposition of negative charges on the molecules' electronegative end allows for the hydrogen to bond with the molecules' negative end. This intermolecular phenomenon enables CQDs to latch on to the bacterial surface thus effecting the fluoresced images. However, the complexity of the structure of *E. coli* due to the phospholipid-peptidoglycan bilayer compared to the single peptidoglycan layer of *S. aureus* offered an added resistance to the penetration of CQDs to the surface.



**Figure 6.** H-bonding as plausible attachment mechanism of CQD to Gram-stained bacteria

#### 4. Conclusion

In conclusion, this study has successfully developed naturally-based quantum dots from glycerol as carbon source. The conjugation of amine groups sourced from TEPA to the CQDs was confirmed by FTIR spectra with the appearance of N-H bending at 1650 to 1580  $\text{cm}^{-1}$ . The maximum excitation or absorbance wavelengths, recorded at 207 and 210 nm, which fall within the UV light range, are suggestive of the  $\pi$ - $\pi^*$  transition of conjugated  $\pi$ -bonds of CQDs. Emission wavelengths peaked at 314.18 nm and 381.15 nm supporting the observed strong blue luminescence under UV lamp which is also an indication of small-sized CQDs formation. As a potential bio-fluorophore, developed CQDs effected more observable fluoresced image of *S. aureus* than that of *E. coli*. This phenomenon was attributed to the complex outer structure of *E. coli* which offered added resistance to the penetration of CQDs via hydrogen bonding as attachment mechanism. For future works, it is intended that the effect of incubation period of CQDs to bacterial models be assessed and the processing conditions be optimized to improve further CQD application as fluorophore.

#### 5. References

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