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Fabrication of Graphene Quantum Dots Based Fluorescent Sensor for Detection of Clenbuterol

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In this work, the graphene quantum dots (GQDs) based fluorescent sensors for detection of clenbuterol (CLB) using the fluorescence resonance energy transfer (FRET) effect were developed and characterized. In the sensors, GQDs played as donor meanwhile the conjugated molecule formed from N-1-naphthylethylen diamine (NED) and diazonium-CLB was the acceptor. A good overlapping between the acceptor's UV-vis spectrum and donor's PL spectrum was observed. The CLB detection limit of the sensors was found at the CLB concentration of 10^{-10} g/ml. A wide linearity relationship between the PL intensities of the sensors and the CLB concentrations in the range from 10^{-4} g/ml to 10^{-10} g/ml was observed. This report contributes to the design of GQDs based fluorescent sensor for detection of growth promoters.

Keywords: Graphene Quantum Dots, FRET, Sensor, Clenbuterol.

1. INTRODUCTION

Clenbuterol (CLB) is one of the artificially synthesized β-agonists, which can promote the animal growth in skeletal muscle mass and decrease the fat accumulation. Unfortunately, the CLB residuals in meat products could lead to potential endangerment of human health and even the occurrence of poisoning incidents.[1–4] Thus, the use of CLB in breeding industry is strictly inhibited since long time. In Vietnam, there are still illegal uses of the growth promoting β-agonist in breeding farms. Therefore, much effort has been devoted to the development of new methods for the detection of CLB. At present, several analytical methods with high limit for detection of CLB residuals are employed such as enzyme-linked immunosorbent assay (ELISA),[5,6] high performance liquid chromatography (HPLC),[7] liquid chromatography coupled with mass spectrometry (LC-MS),[8] gas chromatography coupled with mass spectrometry (GC-MS).[9] However, the draw backs of these methods are extremely time-consuming, requiring skilled staffs and with complicated working steps. It is necessary to develop rapid, convenient and inexpensive methods with high sensitivity for detection of CLB. Recently, we reported the development of fluorescence resonance energy transfer (FRET)-based sensor for rapid detection of CLB.[10] In this sensor, the CdTe quantum dots were used as donor and the acceptor is the conjugated molecules formed by the diazo coupling reaction between diazonium-CLB and NED. However, the CdTe quantum dots showed the photoluminescence at much higher wavelength in comparison with the UV-vis absorption of the acceptor lead to the litter overlapping of these spectra.

In the recent years, graphene quantum dots (GQDs) have attracted much research interests because of the unique and interesting optical, electrical and chemical properties. Compared to conventional semiconductor quantum dots (QDs) such as CdX (X = S, Se, Te), GQDs have low toxicity, chemically inert, biocompatible, and resistant to photobleaching.[11,13] GQDs are promising optical nanomaterials for application in bioimaging[14,15] and bio/sensors.[16] So far, GQDs have been generally prepared by top–down and bottom–up methods. The top–down methods are based on the cutting of carbon nanotubes, graphene sheets or carbon nanofibers to form small

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GQDs.[3, 17] In contrast, by the bottom–up methods small carbon precursors such as hexa-peri-hexabenzocoronene,[18] polyphenylene dendritic precursor,[19] citric acid[20] etc. have been employed to fabricate GQDs. Recently, Wu et al.[14] reported the simple and easy bottom–up method to prepare highly fluorescent GQDs from L-glutamic acid and they are able for application *in vitro/in vivo* imaging and sensing.

In this work, a FRET-based fluorescent sensor for detection of CLB using GQDs as donor and the conjugated-molecules formed from NED and diazonium-CLB as acceptor was developed and characterized. The changes in the fluorescent intensity of the sensor depending on the CLB concentrations were used for determination of the content of CLB in solutions. In our best knowledge, this kind of fluorescent sensor for detection of CLB is firstly reported.

2. EXPERIMENTAL DETAILS

2.1. Chemicals

L-glutamic acid ($C_5H_9NO_4$), Clenbuterol-(4-amino-[(tert-butylamino)methyl]-3,5-dichlorobenzyl alcohol hydrochloride ($C_{12}H_{18}Cl_2N_2O$), N-1-naphthylethylene diaminedihydrochloride ($C_{12}H_{14}N_2 \cdot 2HCl$), sodium nitrite ($NaNO_2$), hydrochloride acid (HCl), sodium hydroxyl (NaOH) were purchased from Sigma Aldrich. All chemicals were used as received.

2.2. Synthesis of the GQDs

The GQDs were synthesized according to the method reported in the literature[14] by pyrolyzing of L-glutamic acid. A glass bottle was heated to 210 °C by a heater. Hence, 1.0 g L-glutamic acid was carefully added into this bottle and the solid L-glutamic acid changed to liquid. When the liquid turned from colorless to brown, formation of GQDs was implied. Then, 20.0 ml water was added into the solution followed by stirring for 30 min. Finally, the mixture cooled to room temperature, the solution was centrifuged at 10.000 rpm for 30 min. The supernatant was collected after centrifuging. The prepared GQDs were stored at room temperature before use.

2.3. Diazotization of CLB

The diazotization of CLB was prepared as the same as in our previous report.[10] 3 mg CLB and 3 ml HCl 0.01 M was placed in a flask and shaken. The flask was then kept in ice bath at temperature of 0–5 °C in dark. Then, 2 ml $NaNO_2$ 0.01 M was added drop wise to the flask. This mixture was stirred vigorously and allowed to maintain for five minutes.

2.4. Preparation of Sensor Solution for Detection of CLB

Firstly, a stock solution (1 μgm^{-1}) of diazonium-CLB was prepared by dissolving an appropriate amount of diazonium-CLB in phosphate buffer (pH = 7.4). The working solutions with different CLB concentrations were made by dilution of stock solution and stored at 4 °C until being used. For each sensor solution, 1 μmol GQDs and 10 mmol NED were injected into a vial. The reaction solution was incubated at 25 °C for 30 min with gentle shaking to immobilize the NED molecules on the GQDs surface. Afterthat, the sensor solution was prepared by mixing of the GQDs immobilized with NED molecules solution and solution of diazonium-CLB.

2.5. Characterization Methods

The photoluminescence measurements of the nanosensors were conducted on HR550 instrument (HORIBA JOBIN YVON) at the Institute of Materials Science (VAST). The UV-vis absorption spectra and FT-IR spectra of the samples were recorded by using a SP-3000 nanospectroscopy and Impact 410 NICOLET at Institute of Chemistry (VAST), respectively. High-resolution transmission electron microscopy (HR-TEM) images were taken on a JEM 2100 (JEOL) at the Institute of Materials Science (VAST).

3. RESULTS AND DISCUSSION

3.1. Characterization of the GQDs

Figure 1 shows HR-TEM image of the prepared GQDs. The GQDs have the diameters from 3 nm to 8 nm. The GQDs' lattice spacing was clearly seen and was measured to be 0.24 nm from the inset picture. The optical properties of GQDs were characterized by UV-vis and PL measurements. Figure 2 shows the UV-vis spectrum and PL-spectrum of the prepared GQDs.

GQDs showed a strong absorption peak at 226 nm and the other weak shoulders at 306 nm. The first absorption peak is assigned to the $\pi - \pi^*$ transition of aromatic C=C bonds and the second absorption peak indicated the size

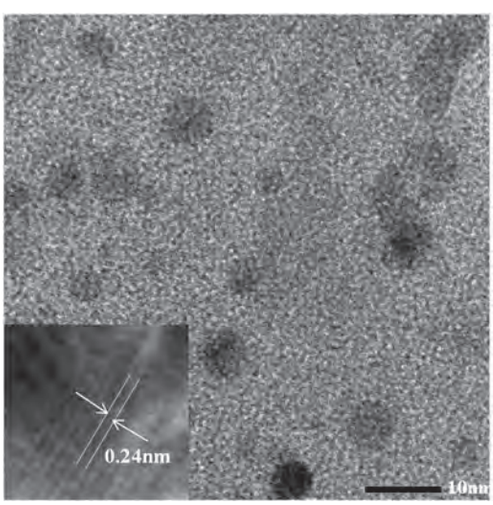

Figure 1. HR-TEM image of the prepared GQDs.

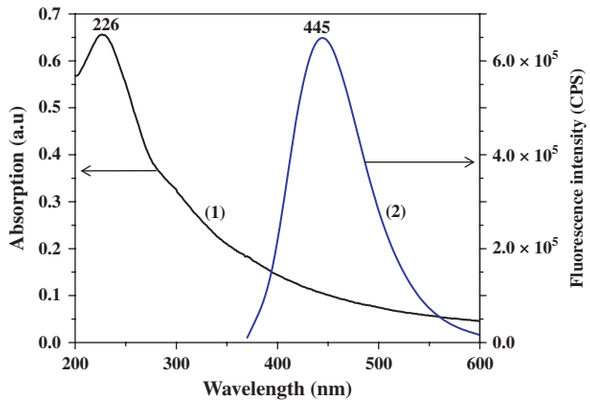

Figure 2. UV-vis spectrum (1) and PL spectrum (2) of GQDs.

uniformity of the sp^2 clusters in the GDQs.[14] Otherwise, GQDs showed a strong emission peak at 445 nm by the exiting wavelength of 360 nm. These results indicate that the optical properties of the prepared QGDs are the same as reported in the literature.[14]

3.2. Working Mechanism of the GQDs Based Fluorescent Sensors for Detection of Clenbuterol

In this work, the working mechanism of the GQDs based fluorescent sensors are based on the FRET effect as the same as reported in our previous work.[10] The prepared GQDs are employed as donor and the conjugated-molecules formed from NED molecules and diazonium-CLB molecules through diazo coupling reaction as acceptor (Fig. 3).

In the sensor structure, NED was used as ligands because this molecule bearing NH_3^+ group that can interact with COO^- groups of GQDs via electrostatic interaction. Otherwise, this molecule can undergo a coupling reaction with diazonium-CLB to form an azo compound that can act as an acceptor in the sensor. The immobilization of NED on the GQDs surface could be proved by comparison of FTIR spectra of the prepared GQDs and the mixture GQDs and NED (Fig. 4).

The GQDs prepared from L-glutamic acid still contain the carboxyl groups on the surfaces.[14] The presence of the carboxyl group on the surface of GQDs is confirmed by the broad peak at 3400 $cm^{-1}$of OH group and the peak of C═O stretching at 1770–1600 cm^{-1} (Fig. 4, curve a). By mixing GQDs and NED, a new peak appears at the wavenumber 3000–2800 cm^{-1}. This peak is ascribed to the electrostatic interaction between the carboxyl group of GQDs and the amine group of NED (Fig. 4, curve b). Comparison to the FTIR spectrum of the mixture of GQDs and NED, the FTIR spectrum of the mixture of GQDs, NED and diazonium-CLB (Fig. 4, curve c) appears the new peak at 1576 cm^{-1}, which indicates the formation of the azo functional group (–N═N–) by coupling reaction between NED and diazonium-CLB.[10] Otherwise, the new peak at 768 cm^{-1} is assigned to the C–Cl stretching of CLB.

TEM image of the GQDs coated by the azo compounds of NED and diazonium-CLB shows the diameters in the range of 15 nm to 20 nm (Fig. 5). This size is bigger than

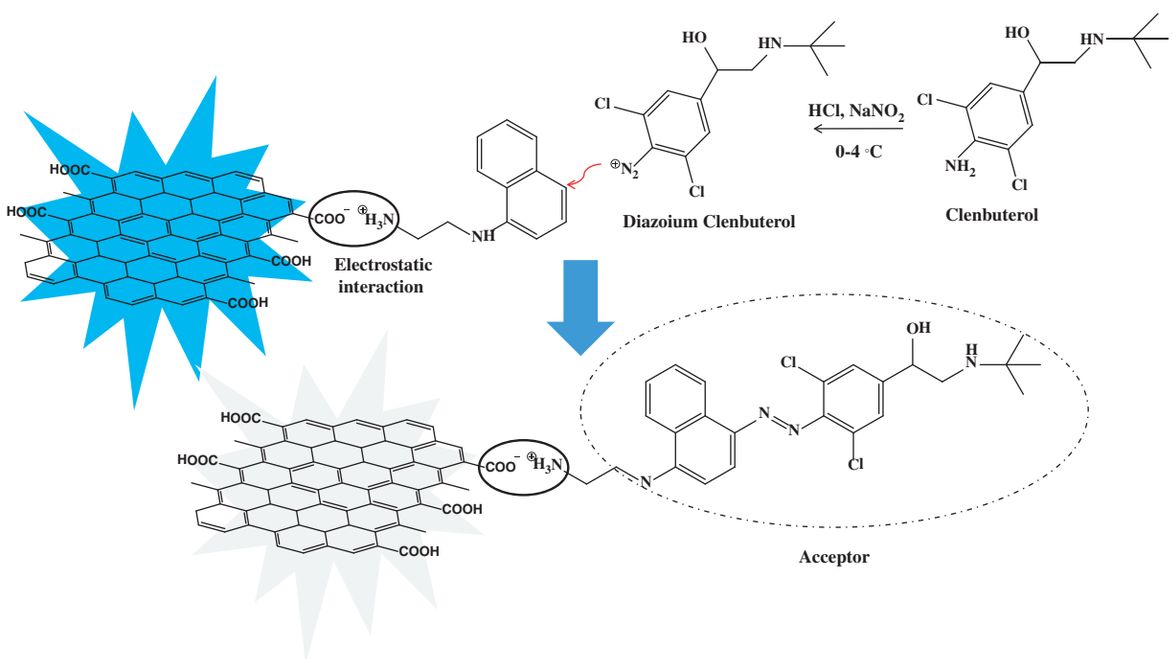

Figure 3. Working mechanism of FRET based sensor.

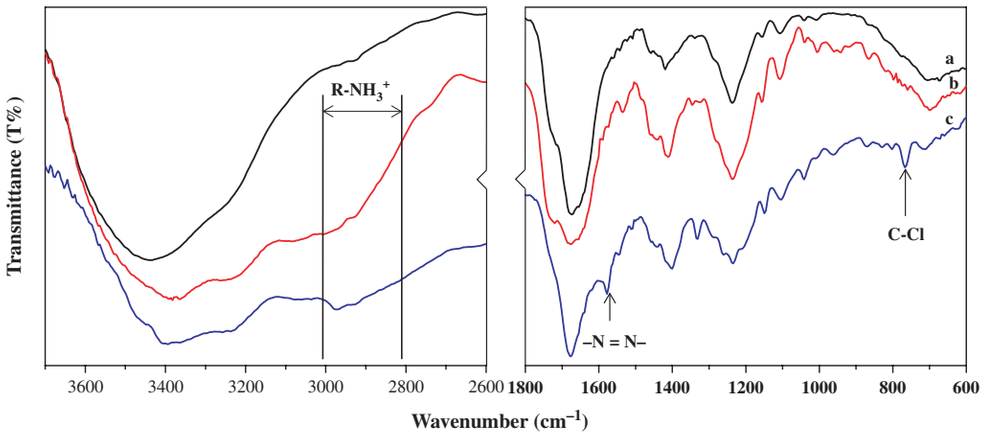

Figure 4. FTIR spectra of GQDs (a), mixture of GQDs and NED (b), mixture of GQDs, NED and diazo-CLB (c).

the size of the as prepared GQDs (Fig. 1). This result could reconfirm the formation of the sensor structure according to the Figure 3.

One of the most important factors to affect the FRET effect is the overlapping of the UV-vis spectrum of the acceptor and the PL spectrum of the donor. Figure 6 shows the UV-vis spectra of CLB, diazonium-CLB and the acceptor formed by diazonium-CLB and NED.

Pristine CLB shows a strong absorption peak at 294 nm. The diazonium-CLB displayed a new peak at 345 nm that attributed by the formation of the diazo group. The azo compounds of NED and diazonium-CLB showed a strong absorption peak at longer wavelength of 464 nm. This new peak exhibited a good overlapping with the PL spectrum of GQDs (Fig. 7).

The maximal emission peak of GQDs is at 445 nm meanwhile the maximal UV-vis absorption peak of the acceptor is at 464 nm. The smaller the difference between these values, the better the overlapping of the UV-vis spectrum of the acceptor and PL spectrum of the donor. In this case, the difference between the wavelength of the maximal UV-vis absorption of acceptor and the wavelength of the maximal emission of GQDs is only 19 nm.

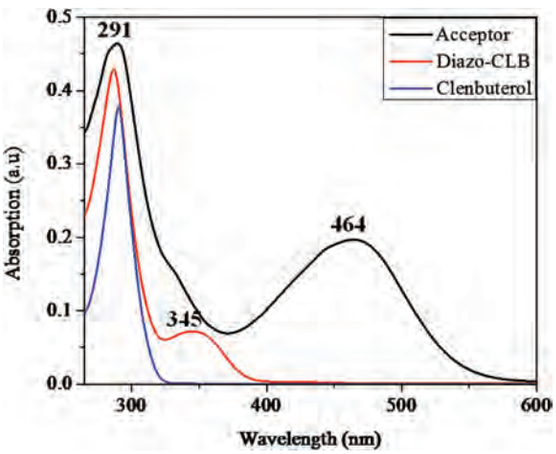

Figure 6. Comparison of UV-vis spectra of CLB, diazonium-CLB and the azo compounds of NED and diazonium-CLB.

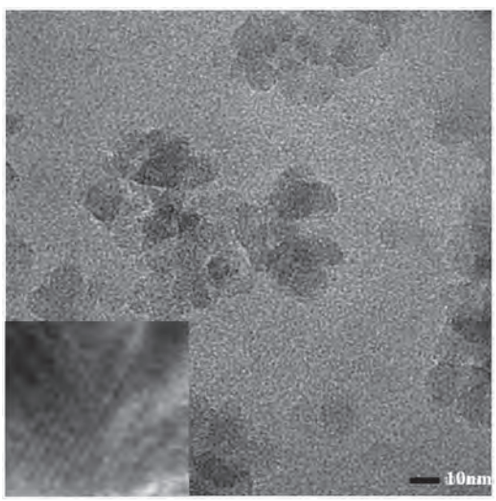

Figure 5. TEM images of the GQDs coated by the azo compounds of NED and diazonium-CLB.

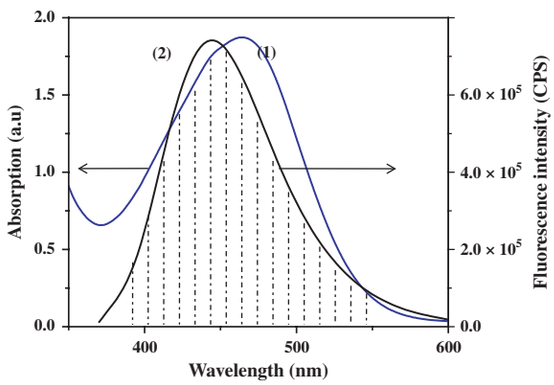

Figure 7. Overlapping of acceptor's UV-vis spectrum (1) and GQDs donor's PL spectrum (2).

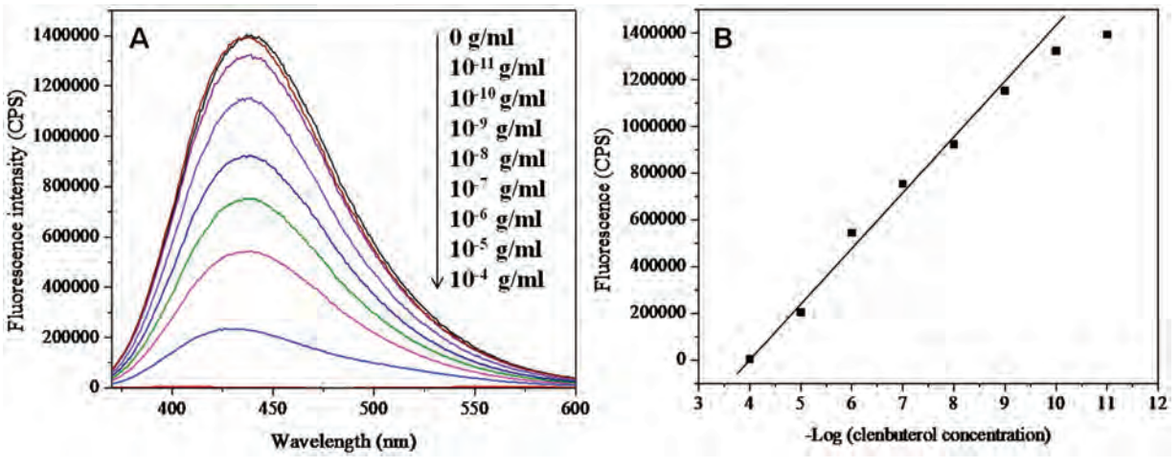

Figure 8. (A) PL spectra of the GQDs in the sensors at different CLB concentrations and (B) relationship between CLB concentrations and the fluorescence intensity of the GQDs.

This result indicates that a good overlapping between the UV-vis spectrum of the acceptor and PL spectrum of GQDs on this sensor structure. Thus, the condition for the FRET effect occurred in the sensor is fulfilled and it could be used for determination of the CLB concentrations.

3.3. Detection Clenbuterol

The PL spectra of GDQs based fluorescence sensor at different CLB concentrations was recorded (Fig. 8(A)). The intensities of PL spectra of GDQs in the sensor decrease with the increasing of CLB concentrations and it is fully quenched at the CLB concentration of 10^{-4} g/ml. At the CLB concentration of 10^{-11} g/ml, the form of the PL spectrum of the sensor is almost identical to the PL spectrum of GQDs. Thus, the detection limit of the sensor is determined at the CLB concentration of 10^{-10} g/ml.

For quantitative determination of CLB concentrations, Figure 8(B) shows the correlation between the PL intensities of the sensors and the negative logarithmic CLB concentrations. A good linearity was observed at the CLB concentrations range from 10^{-4} to 10^{-10} g/ml.

4. CONCLUSION

A GQDs based fluorescent sensors for detection of CLB using FRET effect have been successfully fabricated. The immobilization of the conjugated-molecules formed from NED and diazonium-CLB as acceptor on the surface of GQDs as donor is through the electrostatic interaction between the carboxyl groups of GQDs and the amine group of NED. Because of relative small difference in the wavelength of the maximal absorption peak of the acceptor and the maximal emission peak of the donors, a good overlapping between the acceptor's UV-vis spectrum and donor's PL spectrum was observed. It was shown that the sensitivity of the as-fabricated sensors for detection of CLB was determined at the CLB concentration of 10^{-10} g/ml. For quantitative determination of CLB concentrations, a good linearity relationship between the PL intensities of the sensors and the CLB concentrations at the range from 10^{-4} to 10^{-10} g/ml was found.

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