**Synthesis and Luminescent Properties of Graphene
Quantum Dots (GQDs) for Fluorescence
Quenching by Titanium Dioxide Nanoparticles in
Water Analysis**

by

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Abstract

Graphene quantum dots (GQDs) have many excellent properties of graphene and quantum dots such as strong fluorescence, chemical stability and facile synthesis. In this work, GQDs were prepared by pyrolysis of citric acid. Compared with the conventional pyrolysis method, optimization of the pyrolysis temperature, pyrolysis time, and dispersion pH attained 1.04 times stronger fluorescence emission intensity compared with the previous GQDs.

The appearance of metal oxide nanoparticles in potable water has attracted much public attention concerning their potential adverse health risks. A simple method for quantitative analysis of titanium dioxide (TiO_2) nanoparticles was developed by fluorescence quenching of GQDs in this research. Dopamine (DA) was first added to coat TiO_2 nanoparticles with polydopamine (PDA) under ultrasonication, which broke up all coagulated nanoparticles and prevented any re-aggregation to merit an accurate analysis. GQDs were next added as a fluorescent sensor probe to measure the quenching of its emission intensity by the PDA-coated nanoparticles. Data analysis by the Stern-Volmer equation followed a third-order polynomial fit indicated static, dynamic and absorptive contributions to the total quenching. Detection of TiO_2 nanoparticles down to 0.02 mg/mL was validated, under optimal DA and GQDs concentrations, with a linear dynamic range up to 2.0 mg/mL.

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List of Abbreviations

Citric acid	CA
Energy dispersive X-ray	EDX
Förster resonance energy transfer	FRET
Fourier transform infrared spectroscopy	FTIR
Graphene quantum dots	GQDs
Graphene quantum dots-immobilized polydopamine-coated titanium dioxide	GQDs-PDA-TiO ₂
Phosphate buffered saline	PBS
Polydopamine	PDA
Polydopamine-coated titanium dioxide	PDA-TiO ₂
Transmission electron microscopy	TEM

Chapter 1 Introduction

1.1 Quantum Dots

1.1.1 Quantum dots

Particles with a size ranging from a few to several tens of nanometers are called quasi-zero-dimensional nanomaterial.[1] Quantum dots are a quasi-zero-dimensional nanomaterial [2]. They are called quantum dots since their dimensions are below 100 nanometers (nm), and they appear as tiny points.[3] Quantum dots are commonly spherical and usually made of semiconducting materials.[4]

1.1.2 Quantum confinement

Quantum dots have many unique properties. Once the diameter of the material is less than its de Broglie wavelength of electron wave function, quantum confinement can be observed.[5] The electrons and electron holes of quantum dots are squeezed into one dimension by quantum confinement and the continuous energy state becomes discrete.[6] As a result, quantum dots can emit fluorescence under excitation; both the intensity and wavelength of the emission peak are size dependent.[7] [8]

1.1.3 Fluorescence properties of quantum dots

Based on the quantum confinement effect, the emission peak is narrow (less than 150 nm) and symmetrical, and the fluorescence has a long-term photostability.[9] The fluorescence properties (wavelength and intensity) may change with different semiconducting materials.[10] Changing the size of quantum dots can shift the fluorescence emission spectrum to cover the entire visible region, as illustrated in Figure 1.1.

Figure 1.1 Fluorescence emission of different colors from quantum dots of various particle sizes under the same UV excitation light.

Quantum dots have a broad range of applications such as fluorescent probes, solar cells, light emitting devices, and optical markers.[11] They also have excellent biocompatibility after chemical modification. The cytotoxicity caused by quantum dots is relatively low to humans, so they can be used as a biological marker and cancer probe.[12]

1.2 Graphene Quantum Dots (GQDs)

Graphene is a honeycomb planar sheet formed by sp^2 hybridization of carbon atoms with only one atomic layer thickness of 0.335 nm.[13][14][15][16] It is also one of the carbon allotropes that are shown in Figure 1.2.[17] Physicist A. Geim at the

University of Manchester used a micromechanical stripping method to successfully isolate graphene from graphite, and this won him the 2010 Nobel Prize in Physics.[18] Pure graphene is a zero band-gap semiconductor or semi-metal as shown in Figure 1.3 [19]. The conventional production methods of graphene powder are mechanical stripping, oxidation-reduction, and SiC epitaxial growth, while a graphene film can be produced through chemical vapor deposition.[20]

Figure 1.2 Structure of carbon allotropes: (a) diamond; (b) graphite; (c) lonsdaleite; (d–f) fullerenes (C60, C540, C70); (g) amorphous carbon; (h) carbon nanotube; (i) graphene.[15]

Figure 1.3 The bandgap of insulator, semiconductor, metal and graphene [17].

As graphene has very good electrical, mechanical, optical and thermal properties, it has found numerous engineering applications in aerospace, computer, electronics, and materials.[21] Known as "black gold" to some scientists, graphene has been hailed as being capable of revolutionizing many new technologies in the biological, chemical, environmental and medical fields.[22]

Figure 1.4 Comparison between graphene and graphene quantum dots.

Graphene quantum dots (GQDs) have the same lattice structure as graphene (see Figure 1.4) but only with sheet dimensions of less than 10 nm.[23] GQDs are also honeycombed planar sheets formed by sp^2 hybridization of carbon atoms.[24]

GQDs have both the properties of graphene and quantum dots, such as chemical stability and photostability. One of the properties of GQDs is quantum confinement effect since their internal electrons are limited to specific directions of movement. On account of this, they exhibit great fluorescence emission.[25][26]

GQDs are increasingly being studied by scientists. They have brought revolutionary changes to the fields of biology, environmental science, medicine, etc. and have been used in fluorescence tracers, solar cells, and electronic equipment.[27, 28, 29] Traditional synthesis methods of GQDs include chemical stripping, chemical vapor deposition, electrochemical reduction, and pyrolysis.

1.3 Properties of GQDs

1.3.1 UV-Vis absorption spectrum of GQDs

GQDs usually have strong optical absorption in the ultraviolet region.[30] The π - π^* transition peak is around 200-270 nm, and the n- π transition peak is between 270-390 nm from CO functional groups.[31][32] In Figure 1.5(a), the red line shows the UV-Vis spectrum of GQDs. There is a peak at 320 nm which is from the n- π transition. Due to strong solvent background absorption of UV light, the π - π^* transition peak of GQDs is sometimes difficult to identify in the UV-Vis spectrum.

Figure 1.5 (a) UV-Vis absorption and fluorescence spectra of GQDs dispersed in water; (b) fluorescence excitation and emission spectra.[24]

The optical absorption of GQDs is greatly influenced by their size due to the quantum confinement effect.[33][34][35] When prepared through chemical exfoliation of carbon fibers, the GQDs experience an increase in radius from 1-4 nm to 7-11 nm and a red shift of absorption peak as the synthesis reaction temperature decreases from 120°C to 80°C.[36] The size of the GQDs increases as the synthesis time passes,

Figure 1.6. The GQDs synthesized under different temperatures (120°C, 100°C and 80°C) have three sizes with different UV-Vis spectra. From Figure 1.6(a), the optical absorption spectrum shows a red shift from A to C. It is caused by an increase in the GQDs dimension resulting from a decrease in the synthesis temperature.

Figure 1.6 (a) UV–Vis spectra of GQDs A, B, and C, correspond to synthesis temperature at 120, 100, and 80 °C, respectively. (b) Relationship between energy gap and size of GQDs.[33]

1.3.2 Fluorescence emission spectrum of GQDs

Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation. The mechanism of fluorescence is shown in Figure 1.7. [37]

Figure 1.7 Scheme of mechanism of fluorescence[35].

The fluorescence emission spectrum of GQDs varies from the visible to near infrared region [38][39][40] as related to their atomic structure[41], oxygen functional group[42], single-electron charging effects[43], edge structure effect[44] and free zig-zag sites.[45] The GQDs prepared by different methods exhibit a strong degree of fluorescence behavior.[46] When the size of GQDs increases, the emission spectrum generally exhibits a red shift due to a decreasing HOMO-LUMO band gap, Figure 1.8 [47][48]

Figure 1.8(a) UV–Vis spectra of GQDs A, B and C, corresponding to synthesis temperatures of 120, 100, and 80 °C, respectively; (b) fluorescence emission spectra of GQDs A, B and C.[45]

The fluorescence emission peak wavelength and intensity are often affected by the excitation wavelength.[49] The fluorescence peaks of GQDs experience a redshift as the excitation wavelength increases, showing a significant excitation-dependent photoluminescence behavior. When the excitation wavelength is moved from 320 to 500 nm, the emission peak is shifted from 450 to 550 nm and the maximum emission intensity will decrease significantly, Figure 1.9 (b).[50] The excitation-dependent property of GQDs may be due to their different sizes or different transmission sites, which influence the optical selection differences.[51] The excitation dependency can be eliminated by preparing GQDs of uniform sizes and emission sites.[52]

Figure 1.9 (a) UV-Vis spectrum of GQDs (inset: photographs of GQDs excited in daylight and under 365-nm UV light). (b) Fluorescence emission spectra of GQDs under different excitation wavelengths.[45]

In addition to the size effect and the excitation dependency, the fluorescence intensity of GQDs is also affected by the pH of dispersion in NaOH. In basic environments, GQDs have strong fluorescence; the fluorescence is almost completely quenched in acidic environments, Figure 1.10. [53]

Figure 1.10 pH-dependent fluorescence emission spectra of GQDs when pH is varied between 13 and 1.[51]

In addition, the thickness of GQDs affects their fluorescence intensity. [54] Monolayer GQDs exhibit significant fluorescence while multilayer graphene exhibits a lower fluorescence emission intensity.

1.3.3 Förster resonance energy transfer (FRET) of GQDs

FRET is a mechanism that describes the energy transfer between two fluorescent molecules.[55] When the fluorescence emission spectrum of one fluorescent molecule (also known as a donor fluorophore) overlaps with the excitation spectrum of another fluorescent molecule (also known as a receptor fluorophore), the donor emission energy can induce the receptor to fluoresce at a longer emission wavelength.[56] The fluorescence emission intensity of the donor thereby decreases, Figure 1.11. [57]

Figure 1.11 Schematic comparison between no FRET and FRET.[53]

FRET of GQDs has enormous potential applications in the field of biomedical imaging. In 2015, Bhatnagar *et al.*[58] found a method to diagnose heart attack (myocardial infarction) based on FRET of GQDs to detect a cardiac marker Troponin I (cTnI) in blood. The anti-cardiac Troponin I (anti-cTnI) was covalently conjugated to amino functionalized graphene quantum dots (afGQDs) through a carbodiimide coupling reaction. There was a strong decrease of the fluorescence emission intensity

from GQDs due to FRET between the anti-cTnI/afGQDs and graphene (quencher). With addition of cTnI, graphene was expelled from anti-cTnI/afGQDs to induce the recovery of fluorescence emission intensity. A scheme of the immunosensing is shown in Figure 1.12.

Figure 1.12 Scheme of immunosensing based on interaction of anti-cTnI/afGQDs with graphene.[54]

1.4 Synthesis Methods of GQDs

There are many ways to synthesize GQDs in bottom-up and top-down methods. However, not every method is simple and easy to operate. The size and the fluorescence intensity of the GQDs can be hard to control with some methods. Four common methods of GQDs synthesis are described herein.

1.4.1 Exfoliation and etching method

Acid exfoliation and etching of carbon fibers is a simple method that can synthesize GQDs on a large scale. Carbon fibers are corroded and the debris can be peeled off

by strong acids. In 2012, Peng *et al.*[59] used pitch-based carbon fibers as a raw material and sulfuric acid to peel off layers of graphite which resulted in GQDs. They have zigzag edges and possess the specific electronic or magnetic properties of semiconductors. Their size ranged from 1 to 4 nm; their thickness is around one to three layers of carbon atoms. They have high crystallinity and solubility in water. A scheme for this GQDs synthesis method is shown in Figure 1.13. This method involves a simple reaction step and benefits from the low price of raw material. However, there are still some disadvantages of this method such as nonuniform sizes of the synthesized GQDs.

Figure 1.13 Scheme of GQDs synthesis by exfoliation and oxidative cutting of carbon fibers.[57]

1.4.2 Hydrothermal method

With the development of nanotechnology, the larger size of a graphite thin film limits its direct application to nano-devices. Hence the development of a method to transfer

a thin graphite film into nano-sized GQDs is important. In 2010, Pan *et al.* [60] reported a new hydrothermal method that cut graphite into functionalized GQDs of around 9.6 nm in size. GQDs were obtained by thermal deoxidization of GO sheets in a tube furnace at 200-300°C for 2 hours. The GQDs displayed blue light emission. A scheme for this synthesis method is shown in Figure 1.14. The carboxyl group, epoxy group and carbonyl group were introduced as an acid oxidation of the graphene sheet. During the subsequent hydrothermal reduction, a mixed epoxy chain broke down and the oxygen atoms were removed as shown in the scheme. The hexagonal skeleton stretch was preserved and GQDs were finally formed.

Figure 1.14 Scheme of hydrothermal synthesis of GQDs.[56]

1.4.3 Pyrolysis method

In 2012, Dong [61] *et al.* synthesized GQDs by pyrolysis of citric acid under neutral pH conditions. This is a simple bottom-up method and the degree of citric acid carbonization can be controlled. In this process, citric acid was incompletely pyrolyzed. GQDs were produced as nanosheets that included uniform sp^2 clusters. The GQDs were under 15 nm; the thickness was around 0.5-2 nm. The fluorescence

quantum yield was 9%. The GQDs exhibited blue fluorescence emission ($\lambda_{em,max} = 460-480$ nm) which was excitation-independent. The advantages of pyrolysis method include cheap raw materials and simple reaction process.

1.4.4 Microwave method

In 2012, Tang [62] *et al.* used glucose as a raw material to prepare GQDs by a microwave-assisted hydrothermal process. The GQDs synthesized by this method exhibited good crystallinity, which promised the facile observation of fluorescence. The size of GQDs was around 1.65 nm and emitted deep ultraviolet emission of 4.1 eV. The GQDs exhibited typical excitation-dependent fluorescence properties. The fluorescence quantum yield was around 7-11%.

1.5 Applications of GQDs

1.5.1 Applications for fluorescence property of GQDs

More recent works continue to advance the frontier of science in this booming research field. Numerous bioanalytical sensor designs and biochemical imaging applications speak to the exciting adventures of GQDs,[63] based on their superior biocompatibility and low cytotoxicity.[64] While it can be expected that bioanalytical applications will rapidly evolve, basic research on the fundamental mechanism of GQDs luminescence must also proceed to gain a better insight of their scientific and technological potential.

In 2014, Ju [65] *et al.* synthesized nitrogen-doped GQDs (N-GQDs) from citric acid and dicyandiamide. N-GQDs showed a strong blue fluorescence emission. Because of great sensitivity and selectivity, they can be used as a probe for the detection of glutathione. After addition of Hg^{2+} into the system, bright luminescence from the N-GQDs-Hg system can be successfully applied for imaging the intracellular glutathione in live cells. By means of the reduction of graphene oxide by thermal annealing at 200°C , GQDs with strong green fluorescence emission were prepared from graphene oxide.[66] The GQDs showed low cytotoxicity and the cell activity remained stable.[67] The bright green fluorescence inside the cells indicates translocation of GQDs through the cell membrane, Figure 1.15.

Figure 1.15 Cellular imaging of GQDs fluorescence.[61]

1.5.2 Applications for quenching property of GQDs

Fluorescence quenching is an interesting phenomenon that has been widely utilized in developing fluorescence-based sensors. A novel sensing platform was reported by

Fan *et al.* for the detection of 2,4,6-trinitrotoluene, down to 0.5 $\mu\text{g/mL}$ in solution, by FRET quenching due to π - π stacking interaction with GQDs.[68] The fluorescence quenching between GQDs and gold nanoparticles was studied by Liu *et al.* who observed a gradual decrease of GQDs fluorescence with increasing concentration of gold nanoparticles. This fluorescence quenching obeyed the nonlinear form of Stern–Volmer model, which suggested both static and dynamic quenching.[69] Li *et al.* first quenched the fluorescence of GQDs by mixing with multi-positively charged guanidine-terminated poly amido anime dendrimers to cause aggregation. The addition of highly negatively charged heparin or chondroitin sulfate next resulted in the recovery of the fluorescence because polyamidoanime would prefer to bind with these two target analytes, resulting in the disaggregation of GQDs. Detection limits were as low as 0.02 $\mu\text{g/mL}$ and 0.05 $\mu\text{g/mL}$, respectively, for this fluorescence turn-on assay.[70] Quantitative analysis of Ponceau 4R colorant in solution was achieved by Zhang *et al.* based on the static fluorescence quenching of GQDs. Under optimal pH, temperature, reaction time and GQDs dilution, the detection limit could reach 8 ng/mL.[71]

1.6 Polydopamine

Figure 1.16 Mechanism of dopamine polymerization.[72]

Polydopamine is a dark brown insoluble biopolymer produced simply by autoxidation of dopamine (DA) as shown in Figure 1.16.[73] It coats the surface of all classes of materials (metals, oxides, polymers, liquid/liquid and liquid/air interfaces) with a conformal and robust film [74]. It also provides strong reactivity for secondary functionalization,[75] with nanoparticles.[76] Hence, polydopamine-based coatings are an ideal film forming method for numerous scientific applications.[77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91] A fluorescence sensing strategy for DA detection was developed based on PDA formed on the surface of GQDs.[92] Fluorescent organic nanoparticles composed of an

organic dye and an amphiphilic copolymer were utilized as a fluorescent probe for detecting DA. A significant fluorescence quenching was attributed to the formation of PDA coating on the surface of each fluorescent organic nanoparticles, which lead to photoinduced charge transfer between the organic dye molecules and the PDA.[93] A novel miRNA sensing strategy composed of fluorophore-labeled DNA as probes revealed that the quenching efficiency of mesoporous PDA nanoparticles (70 nm in diameter) could reach 99% at a relatively low PDA nanoparticles concentration.[94]

1.7 Detection of metal oxide nanoparticles

Metal oxide nanoparticles have been used in many fields including industry, medicine, environmental and food science.[95] They can also be used in biosensors and optical sensors for the detection of metal ions. A novel xanthine biosensor was developed based on Fe_3O_4 nanoparticles modified carbon nanotubes. It offers a better analytical performance such as lower detection limit and higher sensitivity compared with non-modified biosensor.[96] CuO and ZnO nanoparticles were synthesized using *Camellia japonica* leaf extract as an inductive and stabilizing agent. They were tested for the optical sensing of metal ions such as Li^+ and Ag^+ and had an excellent analytical performance.[97]

Engineered nanoparticles represent an emerging class of contaminants that are widely distributed in the aquatic environment. The environmental fate and potential health risks of the nanoparticles in the environment have been concerned. The existing contaminants in the environment interact with metal oxide nanoparticles and

decreases bioavailability.[79] The detection of metal nanoparticles has attracted an increasing attention by scientists.

Nanoparticles can be detected by capillary electrophoresis (CE). CE has many excellent features including high separation efficiency, minimal sample and reagent consumption, various separation modes, and low operating cost.[98] A new approach was developed to selectively enhance the ultraviolet (UV) detection sensitivity in CE-UV analysis by interaction with single-stranded DNA.[99] The UV detection sensitivity of ZnO nanoparticles in CE analysis was selectively enhanced after adsorption of dithiothreitol or cysteine [100]

ICP-MS is an element-selective technique. It has excellent mass resolution and extremely high sensitivity for the detection and quantification of metallic ions and metals. Single particle inductively coupled plasma - mass spectrometry (SP-ICP-MS) methods have been reported for the detection of Zn- and Ce-composed nanoparticles in surface and drinking water.[101] SP-ICP-MS methods can also analyze nanoparticle size distributions and determine nanoparticle numbers.[102] However, the SP-ICP-MS technique cannot provide speciation information to distinguish metal oxides from the metal, metal carbonate, metal chloride, and metal sulfide. Our approach to speciation research has long been based on the surface reactivity of nanoparticles. [103,104,105,106]

1.8 Objective of this research work

One objective of this research was to prepare GQDs by pyrolysis of citric acid which is an easy synthetic method to operate and which takes a short time to complete. The

fluorescence property of GQDs was investigated at different pyrolysis temperatures, pyrolysis time, and dispersion pH in order to explore the optimum reaction conditions.

Another objective of this research was to develop a simple and sensitive method with low detection limit for the quantitative determination of metal oxide nanoparticles in water based on the quenching of fluorescence emission from GQDs. The appearance of metal oxide nanoparticles in potable water has attracted much public attention concerning their potential adverse health risks.

1.9 Statement of experimental design

1.9.1 Synthesis of GQDs

In this research, quenching of fluorescence emission from GQDs was applied to the quantitative determination of TiO_2 nanoparticles in water. They are easy to synthesize by using pyrolysis of citric acid - the synthesis process is safe and the raw materials are cheap. They exhibit strong fluorescence emission intensity and can be used as a non-toxic fluorescence probe with good photostability.

1.9.2 Synthesis of GQDs-immobilized PDA- TiO_2 (GQDs-PDA- TiO_2) nanoparticles

In order to develop a quantitative analysis method for TiO_2 nanoparticles using GQDs, interaction between TiO_2 nanoparticles and GQDs would be essential. However, GQDs cannot form a stable complex with TiO_2 nanoparticles directly. Polydopamine was evaluated for use as a linkage between GQDs and TiO_2 nanoparticles to induce fluorescence quenching of GQDs for the quantitatively

analysis of TiO_2 nanoparticles. The feasibility was proven by preparing GQDs-PDA- TiO_2 nanoparticles that could be analysed by FTIR to confirm their macromolecular structure. Using different concentrations of TiO_2 nanoparticles, a Stern-Volmer plot was constructed to explore the quenching mechanism that offered the lowest detection limit.

Figure 1.17 Structure of GQDs-PDA- TiO_2

Chapter 2 Materials and Methodology

2.1 Materials

Aluminum oxide (Al_2O_3) (nanopowder, <50 nm particle size (TEM)), dopamine hydrochloride (DA), titanium (IV) oxide (TiO_2) (nanopowder, 21 nm particle size (TEM), $\geq 99.5\%$ trace metals basis) was purchased from Sigma-Aldrich (Oakville, ON, Canada). Citric acid (CA) was purchased from Fisher Scientific Company (U.S.A.). Phosphate Buffered Saline (PBS, pH=7.3-7.5 at 25°C, 10% water) was purchased from EMD (U.S.A.). Sodium hydroxide (NaOH) was purchased from Caledon Laboratory chemicals (Georgetown, Ont., Canada). Tris(hydroxymethyl)aminomethane (Tris) (>99.9%) was purchased from Aldrich (U.S.A.).

2.2 Instrument

Table 1 Instruments and apparatuses

Instrument	Company (model)
Analytical balance	Ragwag (R2)
Centrifuge	Thermo Scientific (Heraeus Megafuge 8)
Digital orbital shaker	Southwest Science (SBT 300)
Fourier transform infrared spectroscopy	ABB Bomen (MB Series)
Heating mantles	Huanghua Faithful (98-II-C)
Magnetic stirrer	Fisher Scientific (11-500-75)

Oven	Fisher Scientific (Isotemp)
Spectrofluorometer	Horiba Scientific (Fluoro Max-4)
Transmission electron microscopy	FEI Tecnai (G2 F20)
Ultrasonic processor	Ultrasonics (MSK-USP-3N)
UV detector	Bischoff (Lambda 1010)

Horiba FluoroMax-4 spectrofluorometer

The Horiba FluoroMax[®] 4 spectrometer is a powerful tool for measuring fluorescence of solids, liquids, powders and thin films.[] The instrument is easy to use and is controlled by FluorEssence[™] software with Origin[®] embedded for sophisticated data analysis. Users may self-train on this instrument.

Figure 2.1 Horiba FluoroMax-4 spectrofluorometer.

The sample is illuminated by a 150 W xenon, continuous output, ozone-free lamp. The excitation monochromator has an optical range of 220-600 nm blazed at 330 nm. The emission monochromator has an optical range of 290-850 nm blazed at 500 nm. The monochromators can be independently or synchronously scanned for mixtures. The slit widths may be continuously adjusted through the software from 0-30 nm bandpass.

The FluoroMax 4 has two detectors. One is a calibrated photodiode reference detector to correct for intensity and temporal fluctuations in the source during excitation scans. The other detector is an R928P red-sensitive photomultiplier used in photon counting mode with signal linearity up to 2×10^6 counts per second. With a

photon-counting detector, only the signals originating from your sample are measured, noise from the detector is rejected.

The FluorMax 4 allows for a variety of measurement modes: fluorescence, chemi-, bio- and electroluminescence; excitation, emission, synchronous scans; 3D excitation/emission scans, contour mapping, kinetic scans; quantitation and analysis through Origin[®].

The FluorMax 4 includes a single cell sample holder that holds a 1-cm path length (4 mL) cuvette as well as semi-micro cuvettes. A solid sample holder is designed for samples such as thin films, powders, pellets, microscope slides and fibers. The holder consists of a base with a dial indicating the angle of rotation. Solids are measured at an angle of 30° or 60°. For samples that do not fit in the sample compartment, a fiber optic mount and bundles allow for remote sensing of fluorescence. The mount fits in the sample chamber and the fiber optic cables direct the excitation to the sample and the emission back to the instrument. This technique is ideal for remote testing of non-traditional samples like paper, fabrics, skin and hair.

A Quanta-φ integrating sphere can be used to study fluorescence from solid and liquid samples. This sphere has an internal diameter of 6" (15 cm) for high accuracy and precision. The integrating sphere is particularly useful for measuring photoluminescent quantum yields without the need for a complicated apparatus or standards. The Quanta-φ integrating sphere features a bottom loading drawer for solid/powder samples, top-loading center-mounted liquid sample holder that fits standard cuvettes, wiring port for light-emitting devices and electroluminescence and an easy-to-use analysis package for calculating quantum yield and chromaticity. A

combination of the integrating sphere and the photon-counting detector allows for measurement of the absolute photoluminescence quantum yield at any wavelength accessible by the instrument without the need for standards or complex equipment.

2.3 Methodology

2.3.1 Synthesis of GQDs

2g of citric acid (CA) was heated to 180°C. When it became a dark golden liquid, it was added to NaOH solution and changed the pH value from 0 to 14. After 30 minutes of magnetic stirring, the golden dispersion was dialyzed (using a 3500-MWCO cellulose tubing) for 48 hours to obtain pristine GQDs dialysis after small molecules such as citric acid and NaOH escaped.[107] The synthesis mechanism of GQDs is as follows:

Scheme 1 Mechanism of GQDs synthesis from citric acid.

2.3.2 Fourier transform infrared spectroscopy of GQDs

Infrared spectra were measured using a Fourier transform infrared (FTIR) spectrometer (Bomem MB Series, Quebec, Canada). A disc sample was prepared by

grinding 2 mg of GQDs (dried at 80 °C in oven for 12 hours) with 200 mg of spectrophotometric-grade potassium bromide (KBr).

2.3.3 Fluorescence spectroscopy of GQDs

A mixture of GQDs solution (0.2 mL, pH 7), phosphate-buffered saline (PBS) (1.0 mL, 1 M) and distilled deionized water (DDW) (1.0 mL) was added to a quartz cuvette (optical pathway = 1.0 cm). Using the FluoroMax-4 spectrofluorometer with UV excitation light at a wavelength of 365 nm, the emission wavelength was scanned from 370 to 700 nm to obtain a fluorescence spectrum of the GQDs. The slit width was 4 nm for both the emission and excitation wavelengths.

2.3.4 Synthesis of polydopamine-coated TiO₂ (PDA-TiO₂) nanoparticles

TiO₂ nanoparticles (<1.0 g) were dispersed in DDW (60 mL) by ultrasonication for 20 minutes. First dopamine hydrochloride (0.1 g) and next Tris buffer (63 mg) were added to the TiO₂ dispersion. After vigorous magnetic stirring for 3 hours, a dark gray suspension was obtained. The PDA-TiO₂ nanoparticles were collected by centrifugation and washed by DDW 3 times. The clean PDA-TiO₂ nanoparticles were dispersed in DDW (60 mL) for subsequent experiments. The synthesis mechanism of PDA-TiO₂ nanoparticles is as follows:

Scheme 2 Mechanism of PDA-TiO₂ nanoparticles synthesis from TiO₂ nanoparticles and DA.

2.3.5 Synthesis of GQDs-immobilized PDA-TiO₂ (GQDs-PDA-TiO₂) nanoparticles

PDA-TiO₂ nanoparticles dispersion (5 mL) and Tris buffer (5 mg) were added into the GQDs solution (10 mL). After vigorous magnetic stirring (for 12 hours), a dark yellow dispersion of GQDs-PDA-TiO₂ nanoparticles was obtained. The synthesis mechanism of GQDs-PDA-TiO₂ nanoparticles is as follows:

Scheme 3 Mechanism of GQDs-PDA-TiO₂ nanoparticles synthesis from GQDs and PDA-TiO₂ nanoparticles.

2.3.6 Analysis of GQDs-PDA-TiO₂ nanoparticles by transmission electron microscopy (TEM)

Two drops of GQDs-PDA-TiO₂ nanoparticles suspension were deposited on a sample holder. The sample was dried at room temperature (for 2 hours) before analysis by an FEI Tecnai G2 transmission electron microscope (TEM) (Hillsboro, OR, USA) operated at an accelerating voltage of 120-200 kV.

2.4 Fluorescence spectroscopy of GQDs-PDA-TiO₂ nanoparticles

A mixture of GQDs-PDA-TiO₂ nanoparticles dispersion (0.2 mL), PBS (1 mL) and DDW (1 mL) was added to a quartz cuvette. Under excitation with UV light at a wavelength of 365 nm, the fluorescence emission spectrum was scanned from 370 to 700 nm. The slit width was 4 nm for both the emission and excitation wavelengths.

Chapter 3 Results and Discussion - Properties of GQDs

3.1 Characterization of GQDs

Citric acid was used as a source of carbon in the synthesis of GQDs by pyrolysis of its -COOH functional groups. Under a high temperature of $160\text{-}220^\circ\text{C}$, an intermolecular dehydration reaction occurs among the citric acid molecules (as shown in Figure 3.1). The size of GQDs synthesized by this method is typically 15 nm, and the thickness is 0.5 to 2 nm.[59]

Figure 3.1 Synthesis of GQDs by pyrolysis of citric acid under a range of temperatures.

3.1.1 FTIR analysis

FTIR spectroscopy was performed to characterize the synthesized GQDs. As shown in Figure 3.2, GQDs exhibit a characteristic peak at 3423 cm^{-1} , which is ascribed to the hydroxyl (-OH) groups linked to the honeycomb planar sheet. A characteristic peak at 1579 cm^{-1} is ascribed to $\text{C}=\text{C}$ hexagonal skeleton stretch. Another characteristic peak at 1398 cm^{-1} is attributed to the vibrational peak of COO^- . Lastly, a characteristic peak at

1297.3 cm^{-1} is ascribed to carboxylic stretching (C-O) [108], and 1079 cm^{-1} is attributed to C-O-C, both of which confirm the pyrolysis of citric acid.

Figure 3.2 FTIR spectrum of GQDs.

3.1.2 TEM analysis

TEM was next performed to examine the morphology and size distribution of GQDs. The image in Figure 3.3 clearly shows that GQDs are spherical in shape. The GQDs nanospheres display a resulting clean and structure homogenized microsphere. The three quantum dots marked in red had a diameter of 5.21 nm, 5.34 nm, and 5.24 nm. Most of the particles are not agglomerated and the size of GQDs synthesized by the pyrolysis method turned out to be uniform. Their sizes are mostly less than 10 nm and this meets the size range for quantum dots.

Figure 3.3 TEM image of GQDs.

3.2 Fluorescence analysis

The prepared GQDs were highly fluorescent due to the extended π -conjugation structure of carbon atoms in the honeycomb lattice. They exhibit excellent fluorescence emission due to both quantum confinement and boundary effect. As shown in Figure 3.4, under excitation by light with a wavelength of 365 nm, the GQDs showed a strong emission intensity at the wavelength of 465 nm. This photoluminescence spectrum was independent of the excitation wavelength, indicating a fairly uniform nanoparticle size for all the GQDs.

Figure 3.4 Fluorescence emission (using $\lambda_{\text{ex}} = 365 \text{ nm}$) and excitation (using $\lambda_{\text{em}} = 465 \text{ nm}$) spectra of GQDs.

3.2.1 Influence of reaction time on GQDs fluorescence

Figure 3.5 Two steps of the GQDs synthesis method.

The synthesis of GQDs can be separated to step 1 and step 2 as shown in Figure 3.5.

The reaction time for step 1 and 2 and the relevant FL intensity is shown in Figure 3.6 and Figure 3.7.

Figure 3.6 Fluorescence intensity for different reaction times in step 1. (The relevant average sizes of GQDs are 2, 5, 10, 20 nm.)

Figure 3.7 Fluorescence intensity for different reaction times in step 2.

At 8 minutes, the color changed to dark golden. After 8 minutes, the liquid became viscous, and its color changed to black. After 15 minutes, the color turned black to form a sticky solid. Figure 3.6 shows the fluorescence intensity increase as increasing the reaction time of step 1. Figure 3.6 shows how fast the fluorescence emission intensity increased with increasing pyrolysis time. This indicates when the pyrolysis time was short, citric acid was not completely decomposed to form GQDs. After 8 minutes, the sample became black and sticky; it might form larger nanoparticle such as graphene oxide.[109] The relevant TEM images for the GQDs synthesized under different pyrolysis times are shown in Figure 3.8. With a longer pyrolysis time, the pyrolysis continued to form larger GQDs. In step 2, the pyrolysis liquid was poured

into a NaOH solution. The time allowed between 10 and 180 minutes had little effect on the fluorescence intensity from GQDs, as shown in Figure 3.7.

Figure 3.8 TEM images of GQDs synthesized under different pyrolysis times: (a) 2 min; (b) 4 min; (c) 6 min; (d) 8 min.

3.2.2 Influence of temperature on GQDs fluorescence

From the discussion above, step 1 is a key step of pyrolysis. The pyrolysis temperature in step 1 was varied to obtain a series of fluorescence emission spectra for presentation in Figure 3.9.

Figure 3.9 Emission spectra of GQDs synthesized under different reaction temperatures in step 1. (The relevant average sizes of GQDs are 2, 5, 15, 40 nm.)

As the pyrolysis temperature was changed from 160°C to 220°C, the emission of GQDs varied in both peak wavelength and maximum intensity as shown in Figure 3.9. This variation of peak wavelength can be explained by the size of GQDs produced. The corresponding TEM images for the GQDs synthesized under different pyrolysis temperatures are shown in Figure 3.10. As the pyrolysis temperature was increased, the size of synthesized GQDs increased as well. This induced a redshift of fluorescence emission spectra as well as a increment of the maximum fluorescence intensity. Under the quantum confinement effect, when the size of GQDs reaches the Bohr radius of exciton, the electron energy level near the Fermi level changes from

quasi-continuous to discrete.[110] When the size of GQDs increases, the emission spectrum generally exhibits a red shift due to a decreasing HOMO-LUMO band gap.[111][112] Obviously, the highest emission intensity was obtained from a pyrolysis temperature of 200 to 220°C.

Figure 3.10 TEM images of GQDs synthesized under different pyrolysis temperatures: (a) 160°C; (b) 180°C; (c) 200°C; (d) 220°C.

3.2.3 Influence of pH on GQDs fluorescence

The fluorescence emission intensity was measured, with an excitation wavelength of 365 nm, for different NaOH concentrations (pH 0-14) used in step 2.

Figure 3.11 Fluorescence intensity for different pH values of NaOH solution.

As shown in Figure 3.11, the fluorescence property of GQDs depended on the different NaOH concentrations (pH 0-14) used in the second step of the synthesis. Maximum emission intensity is attained at pH 8 which is likely related to carboxyl groups on the surface of GQDs. When the solution pH is low, the carboxyl functionality is $-\text{COOH}$ which is not charged. Under this condition, the GQDs interact favorably with electrons in the solution. The non-radiative transition is strong, and the radiative transition is weak. Consequently, the fluorescence emission intensity is reduced at low pH levels. At high pH levels, the $-\text{COO}^-$ groups form on the GQDs (and may even fall off due to hydrolysis), resulting in a decrease in fluorescence intensity.

3.3 Conclusion

In this chapter, citric acid was used as a source of carbon in the synthesis of GQDs by pyrolysis of its –COOH functional groups. FTIR spectroscopy was performed to characterize the synthesized GQDs and TEM was next performed to examine the morphology and size distribution of GQDs. The optimized synthesis condition was obtained under the pyrolysis temperature of 200 °C, pyrolysis time of 8 min and dispersion pH of 8.

The effects of reaction parameters on fluorescence property of GQDs are shown as following:

- (1). Fluorescence emission intensity of GQDs increased with increasing pyrolysis time. The maximum of FL intensity is reached at 8 minutes. After that, larger nanoparticle such as graphene oxide might be formed.
- (2). The pyrolysis temperature affects the emission spectrum. As increasing the pyrolysis temperature, the spectrum exhibits a red shift and an incensement of emission intensity. This indicates that GQDs is size-dependent.
- (3). The pH of dispersion effects emission intensity of GQDs. Maximum emission intensity is attained at pH 8. The fluorescence emission intensity is reduced at low pH levels.

3.4 Connection to Chapter 4

Chapter 4 will use the GQDs synthesized by the optimized pyrolysis method in this chapter to synthesize GQDs-PDA-TiO₂ nanoparticles. Fluorescence quenching of

GQDs will be used to develop a new method for quantitative detection of TiO_2 nanoparticles in water in Chapter 4.

Chapter 4 Results and Discussion- Characterization of GQDs-PDA-TiO₂

4.1 Morphology

TEM images of PDA-TiO₂ nanoparticles obtained in the process of ultrasonication treatment and magnetic stirring of dopamine and TiO₂ are shown in Figure 4.1 (a) and (b). Corresponding particle size distribution is presented in Figure 4.1 (c). The average particle size of PDA-TiO₂ nanoparticles was found to be around 25-35 nm. It can be noticed that the thin coating of PDA formed on the surface of TiO₂ nanoparticles.

Figure 4.1 (a-b) TEM images of PDA-TiO₂ nanoparticles. (c) Corresponding size distribution of PDA-TiO₂ nanoparticles.

TEM was also employed to examine the morphology and size distribution of the GQDs-PDA-TiO₂ nanoparticles. The images in Figure 4.2(a,b) show that these nanoparticles have an average size of 40 nm approximately. Figure 4.2 (c) shows an approximately 4-nm thick film coating the surface of each TiO₂ nanoparticle.

In addition, energy dispersive X-ray (EDX) spectroscopy was used to analyze the composition of these GQDs-PDA-TiO₂ nanoparticles. A typical EDX spectrum is illustrated in Figure 4.3 to reveal the main elements to be carbon, oxide, and titanium. At 0.3 keV on the X-ray energy scale, there is a strong carbon peak as supporting evidence that the TiO₂ nanoparticles were encapsulated by a film of PDA with immobilized GQDs. At 0.5 keV, there is a strong oxide peak to suggest TiO₂ and GQDs. It may be summarized that GQDs-PDA-TiO₂ nanoparticles were successfully

synthesized. There are small peaks of sodium (coming from the NaOH added to adjust pH during the synthesis of GQDs) and copper (coming from the copper-grid sample holder). These impurities are relatively minor, as indicated already at the upper right corner to be Cu (6.2 Wt%), Na (5.7 Wt%), Si (1.8 Wt%) and Al (0.1Wt%).

Figure 4.2 (a)(b)(c) Transmission electron microscopy (TEM) images of GQDs-PDA-TiO₂ nanoparticles, (d)TEM image of TiO₂ nanoparticles.

Figure 4.3 Energy dispersive X-ray (EDX) spectrum of GQDs-PDA-TiO₂ nanoparticles.

4.2 Chemical structure

4.2.1 PDA-TiO₂ nanoparticles

Due to the large specific surface area of TiO₂ nanoparticles and number of Ti atoms in the surface, the coordination of the surface atoms is insufficient. Both unsaturated bonds and dangling bonds exist, with large surface energy and high surface activity to combine easily with other atoms. Under the weakly basic condition in Tris buffer, dopamine (DA) self-polymerized by auto-oxidation [113] to form PDA that firmly

adhered to the surface of solid TiO₂ nanoparticles by chemisorption, as shown in Figure 4.4. Around the TiO₂ nanoparticles, PDA formed a good coating under ultrasonication which breaks up all coagulated nanoparticles and prevents any re-aggregation to merit an accurate analysis. The PDA coating could subsequently facilitate binding interaction with GQDs to enhance static quenching of their fluorescence emission intensity.

Figure 4.4 (a) Preparation and (b) structure of PDA-TiO₂ nanoparticles

This was verified by FTIR spectroscopy, as shown in Figure 4.5 (a) and 4.5 (b), for TiO₂ nanoparticles before and after PDA coating. TiO₂ exhibits a characteristic peak at 1630 cm⁻¹, which is ascribed to the bending mode of TiO₂ nanoparticles.[114] Another characteristic peak at 3433 cm⁻¹ corresponds to the stretching vibration of the O-H group of the TiO₂ nanoparticles. The broad strong peak that spans across the 400-900 cm⁻¹ region in Figure 4.5 (a) is ascribed to Ti-O-Ti. In Figure 4.5 (b), PDA-TiO₂ nanoparticles exhibit additional characteristic peaks at 1491, 1313 and 1261 cm⁻¹. The N-H stretching vibration of PDA appears at 3100-3500 cm⁻¹ which overlap with the O-H stretching vibration to produce a broad peak. This spectrum proves that PDA was firmly adhered to the surface of TiO₂ nanoparticles. As DA is soluble in

water, it stayed in the supernatant after centrifugation and was easily washed away from the PDA-TiO₂ nanoparticles with water. Although PDA has been applied in a manifold way, its structure is still under discussion as reported by Liebscher and co-workers who assigned a chemical structure of dihydroxyindole and indoleione units with different degrees of unsaturation that are covalently linked by C–C bonds between their benzene rings.[115]

Figure 4.5 (a) FTIR spectrum of PDA-TiO₂ nanoparticles.

Figure 4.5 (b) FTIR spectrum of TiO₂ nanoparticles.

4.2.2 GQDs-PDA-TiO₂ nanoparticles

GQDs could be immobilized on PDA-coated TiO₂ nanoparticles, as illustrated in Figure 4.6. FTIR spectroscopy was used to characterize the GQDs-PDA-TiO₂ nanoparticles. Comparing Figure 4.7 with Figure 4.5 (b), the extra peaks are 1384 cm⁻¹ ascribed to C-OH, 1167 cm⁻¹ ascribed to C-C, and 1102 cm⁻¹ ascribed to C-O-C, all of which confirm the presence of GQDs. The structural property of GQDs has recently been reviewed by Choi [116]. As discussed above, the characteristic peak at 1633 cm⁻¹ confirms the presence of PDA-TiO₂ nanoparticles.

Figure 4.6 Structure of GQDs-PDA-TiO₂ nanoparticles.

Figure 4.7 FTIR spectrum of GQDs-PDA-TiO₂ nanoparticles.

4.3 Fluorescence property of GQDs-PDA-TiO₂ nanoparticles

4.3.1 Fluorescence intensity trends of GQDs-PDA-TiO₂ nanoparticles

In order to study the effect of TiO₂ on the fluorescence property of GQDs-PDA-TiO₂ nanoparticles, a series of samples were prepared using different concentrations of PDA-TiO₂ nanoparticles in the same volume of GQDs as summarized in Table. All of the samples were magnetically stirred for 12 hours at a room temperature of 21±1°C.

Table 2 GQDs-PDA-TiO₂ nanoparticles samples prepared using different concentrations of PDA-TiO₂ nanoparticles.

Sample No.	1	2	3	4	5	6	7	8	9
GQDs(mL)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
PDA-TiO ₂ (mg/mL)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Sample No.	10	11	12	13	14	15	16	17	
GQDs(mL)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
PDA-TiO ₂ (mg/mL)	4.5	5.0	6.0	8.0	10.0	12.0	14.0	16.0	

Figure 4.8 demonstrates how, under excitation by UV light at a wavelength of 365 nm, the fluorescence emission intensity of GQDs-PDA-TiO₂ nanoparticles decreased as the concentration of PDA-TiO₂ nanoparticles was increased, together with a small shift of the emission spectrum towards the red region. The reduction of fluorescence emission peak intensity is presented. The reduction of fluorescence emission peak intensity is presented in Figure 4.9 to indicate how the increasing concentration of

PDA-TiO₂ nanoparticles decreased the fluorescence emission intensity of GQDs-PDA-TiO₂ nanoparticles. One plausible explanation for the decreased emission intensity is fluorescence quenching due to photoinduced charge transfer between GQDs and PDA-TiO₂ nanoparticles which may be considered as a quencher.

(a)

(b)

Figure 4.8 (a) Fluorescence emission spectrum of GQDs stock. (b) Fluorescence emission spectra of GQDs-PDA-TiO₂ samples prepared from different concentrations of PDA-TiO₂ nanoparticles.

Figure 4.9 Fluorescence emission intensities of GQDs-PDA-TiO₂ samples prepared from different concentrations of PDA-TiO₂ nanoparticles.

4.3.2 Stern-Volmer plot for GQDs-PDA-TiO₂ nanoparticles

Decreases in the fluorescence emission intensity from GQDs is conventionally quantified by the Stern-Volmer equation:

$$I_0/I = 1 + K_{SV}[Q]$$

where I_0 and I are the fluorescence emission intensity before and after the addition of a quencher, respectively. K_{SV} is the Stern-Volmer quenching constant (mL/mg), and $[Q]$ is the quencher concentration (mg/mL). In general dynamic quenching, the plot of I_0/I versus $[Q]$ is linear, and K_{SV} can be derived from the slope.

Using the fluorescence intensity of GQDs for I_0 , the fluorescence intensity of GQDs-PDA-TiO₂ nanoparticles for I , and the concentration of PDA-TiO₂ nanoparticles for $[Q]$, the Stern-Volmer plot presented in Figure 4.10.(b) was constructed to be linear below 2 mg/mL of PDA-TiO₂ nanoparticles. A third-order polynomial trend line was needed to fit all the data points up to 16 mg/mL of PDA-TiO₂ nanoparticles with a good R^2 value of 0.999, as shown in Figure 4.10. (a).

(a)

(b)

Figure 4.10 Stern-Volmer plot of GQDs-PDA-TiO₂ nanoparticles.

Such deviation from linearity for all concentrations of PDA-TiO₂ nanoparticles higher than 2.5 mg/mL strongly suggests that three independent mechanisms were responsible for quenching the fluorescence emission intensity from GQDs. [117] The first mechanism, dynamic quenching, was due to biomolecular interaction at a distance without direct contact. The collision with PDA-TiO₂ nanoparticles induced a non-radiative transition from the excited electronic state down to the ground electronic state of GQDs. The slope of the straight line, observed for data points below 2 mg/mL of PDA-TiO₂ nanoparticles, represents the dynamic quenching constant. The second mechanism, static quenching, results from the formation of a stable complex due to binding between GQDs and PDA-TiO₂ nanoparticles, followed

by fluorescence resonance energy transfer. It is important to recognize that a linear Stern-Volmer plot does not prove that collisional quenching of fluorescence has occurred because static quenching may also result in a linear Stern-Volmer plots. Static and dynamic quenching can be distinguished by their differing dependence on temperature and viscosity, or preferably by lifetime measurements [118]. The third mechanism is the absorption of excitation and emission light by PDA-TiO₂ nanoparticles, as evidenced by the UV-visible spectrum in Figure 4.11. It shows a high absorbance at the excitation wavelength of 365 nm and a moderate absorbance at the emission wavelength of 465 nm. Thus the fluorescence emission intensity of GQDs could be quenched.

Figure 4.11 UV-Vis spectrum of PDA-TiO₂ nanoparticles dispersed in water.

4.3.3 Quantitative analysis of TiO₂ nanoparticles in synthetic water sample

In order to validate the detection limit of this method, a series of GQDs-PDA-TiO₂ nanoparticles samples were prepared by using low concentrations (0.005 to 0.1 mg/mL) of TiO₂ nanoparticles with 0.5 mg/mL of DA (Table 4) to synthesize PDA-TiO₂ nanoparticles before addition of a fixed quantity of GQDs. From the Stern-Volmer plot presented in Figure 4.12, PDA-TiO₂ nanoparticles proportionally quenched the fluorescence emission intensity of GQDs down to a detection limit of 0.02 mg/mL.

Table 3 GQDs-PDA-TiO₂ nanoparticles samples prepared using low concentrations of PDA-TiO₂ nanoparticles.

Sample No.	1	2	3	4	5	6	7	8
GQDs(mL)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
PDA-TiO ₂ (mg/mL)	0.005	0.01	0.015	0.02	0.025	0.03	0.04	0.05

Sample No.	9	10	11	12	13
GQDs(mL)	5.0	5.0	5.0	5.0	5.0
PDA-TiO ₂ (mg/mL)	0.06	0.07	0.08	0.09	0.1

Figure 4.12 Stern-Volmer plot of GQDs-PDA-TiO₂ nanoparticles at low concentrations.

In order to further investigate the analytical sensitivity at lower concentrations of TiO₂ nanoparticles, the concentration of dopamine was increased from 0.1 mg/mL to 0.5 mg/mL and 1.0 mg/mL. As shown in Figure 4.13, a significant enhancement of the quenching sensitivity was attained by increasing the dopamine concentration to 1.0 mg/mL to afford a lower detection limit of 0.015 mg/mL TiO₂ nanoparticles. The Stern-Volmer quenching constant increased from 0.0419 to 1.0903. The constant 1.0903 could be used to calculate the concentration of TiO₂ nanoparticles when their concentration is larger than 0.015 mg/mL. One plausible explanation for the enhancement of quenching sensitivity is that dopamine itself is also a fluorescent substance.[119] As the emission wavelength of GQDs is nearly the same as the

excitation wavelength of dopamine, dopamine will absorb the photons emitted from GQDs via fluorescence resonance energy transfer. The fluorescence spectrum and the FRET mechanism were shown in Figure 4.14. As I_F decreases, I_F^0/I_F increases, and the quenching is enhanced for more sensitive quantitative analysis of TiO_2 nanoparticles down to 0.01 mg/mL. These results are similar to the linear response of fluorescence quenching of GQDS recently reported by Zhang *et al.* for quantitative analysis of Ponceau 4R in a range of 0.005–0.15 mg/mL.[39] Their low detection limit could reach 8 ng/mL by using more diluted GQDs.

Figure 4.13 Stern-Volmer plot of GQDs-PDA-TiO₂ nanoparticles for different concentrations of DA.

Figure 4.14 (a) Excitation and emission spectra of dopamine; (b) FRET mechanism diagram.

Another factor is the amount of GQDs. As the volume of GQDs was varied from 100 to 10 μL for the quantitative analysis of 0.01, 0.02, 0.05 and 1.0 mg/mL of PDA-TiO₂ nanoparticles, four series of I_F^0/I_F data points were obtained for the Stern-Volmer plot. As presented in Figure 4.15, the I_F^0/I_F data points spread out widely when 10 μL of GQDs or less was used. This means the four concentrations of PDA-TiO₂ nanoparticles had a significantly different effect on quenching the fluorescence emission from GQDs. With these small amounts of GQDs, static quenching due to the formation of stable GQDs-PDA-TiO₂ nanoparticles became dominant. Dynamic quenching was reduced as free GQDs were less available. This combination effectively enhanced the analytical sensitivity for better differentiation of low PDA-TiO₂ nanoparticles concentrations. Where 2 μL of GQDs was the smallest amount used, 0.01 mg/mL of PDA-TiO₂ nanoparticles was easily distinguished from 0.002 mg/mL of PDA-TiO₂ nanoparticles as evidenced by the leftmost data points.

Figure 4.15 Stern-Volmer plot of GQDs-PDA-TiO₂ nanoparticles for different volumes of GQDs.

For stability assessment, one water sample was spiked with GQDs-PDA-TiO₂ nanoparticles for comparison with another water sample was spiked with GQDs. Both were analyzed every 8 hours over three days. As shown in Figure 4.16, their fluorescence intensities decreased only slightly with time. Hence the method could be adopted for environmental water analysis after proper sample treatment with DA and GQDs.

Figure 4.16 Stability assessment of GQDs and GQDs-PDA-TiO₂ nanoparticles in water samples.

Last, standard solutions containing Al₂O₃ nanoparticles were analyzed using the newly developed method. As indicated by the slopes of Stern-Volmer plot in Figure 4.17, the K_{SV} of Al₂O₃ is ten times lower than that for TiO₂ nanoparticles. One plausible reason for this discrepancy can be attributed to the catalytic oxidation property of TiO₂. Thus TiO₂ nanoparticles can be determined in the presence of Al₂O₃ nanoparticles without significant interference. No other substances that might interfere with the detection of TiO₂ nanoparticles were studied because only nanoparticles can be pre-concentrated by centrifugation of the water sample.

Figure 4.17 Stern-Volmer plots for GQDs-PDA-TiO₂ nanoparticles and GQDs-PDA-Al₂O₃ nanoparticles.

4.4 Conclusion

This chapter mainly introduces a method for the synthesis of GQDs-PDA-TiO₂ nanoparticles, the fluorescence properties of GQDs-PDA-TiO₂ nanoparticles, and the application of GQDs-PDA-TiO₂ nanoparticles for the quantitative measurement of TiO₂ nanoparticles in water. Firstly, dopamine self-polymerized by auto-oxidation to form PDA that firmly adhered to the surface of solid TiO₂ nanoparticles by chemisorption. The PDA coating served as a linkage that combined GQDs with TiO₂ nanoparticles to form GQDs-PDA-TiO₂ nanoparticles. The structure of GQDs-PDA-TiO₂ nanoparticles was verified by FTIR spectroscopy. TEM images clearly showed

that TiO₂ nanoparticles were encapsulated by a coating of PDA with immobilized GQDs.

Through the fluorescence analysis of GQDs-PDA-TiO₂ nanoparticles, the following conclusions were obtained:

1. The increasing concentration of PDA-TiO₂ nanoparticles decreased the fluorescence emission intensity of GQDs-PDA-TiO₂ nanoparticles. The plausible explanation for this phenomenon is the fluorescence quenching of GQDs by PDA-TiO₂ nanoparticles. Stern-Volmer plot of GQDs-PDA-TiO₂ nanoparticles which was a third-order polynomial trend line indicated three independent mechanisms of fluorescence quenching. They are static, dynamic and absorptive contributions to the total quenching.
2. The quantitative detection of TiO₂ nanoparticle in water was developed by using the fluorescence quenching GQDs-PDA-TiO₂ nanoparticles. The detection limit is 0.020 mg/mL. A lower detection limit of 0.015 mg/mL TiO₂ nanoparticles was attained by increasing the dopamine concentration to 1.0 mg/mL.
3. TiO₂ nanoparticles can be determined in the presence of Al₂O₃ nanoparticles without significant interference.

Chapter 5 Final Conclusion and Future Work

5.1 Final conclusion

Quenching of GQDs fluorescence has been developed into a simple method for the determination of TiO₂ nanoparticles in water analysis. Synthesis of GQDs was cost-effective at an optimal temperature of 200°C, NaOH solution at pH 8, and pyrolysis time of 8 minutes. They served as a good fluorophore for quenching by TiO₂ nanoparticles in water analysis after treatment with DA. A PDA coating around TiO₂ nanoparticles facilitated the static quenching of fluorescence emission from GQDs. FTIR spectroscopy confirmed the chemical composition of GQDs-PDA-TiO₂ nanoparticles and TEM imaging verified their morphology. Emission spectra from different PDA-TiO₂ nanoparticles concentrations validated the fluorescence quenching as a quantitative analysis method that offers a detection limit adequate for 0.02 mg/mL TiO₂ nanoparticles. Lower detection limits (≤ 0.01 mg/mL) were attainable after maximizing the concentration of DA and minimizing the amount of GQDs. Under optimal DA and GQDs conditions, the dynamic range was linear up to 2.0 mg/mL. Interference by Al₂O₃ nanoparticles was demonstrated to be insignificant. The study is underway to evaluate how selective the method is for TiO₂ in the presence of other metal oxide nanoparticles. Selectivity will also be assessed across a broader variety of nanoparticles including carbon, metals, and polymers. As drug delivery applications for TiO₂ nanoparticles are envisaged, an exposure risk to the public exists beyond their accumulation in the organs and tissues of occupational groups [120]. More biokinetic studies at the low single dose level (typically 40–400 $\mu\text{g/kg}$ body weight) can potentially benefit from a simple analytical method that

requires only the availability of a lab spectrofluorometer, cost-effective preparation of GQDs, and commercially available DA.

5.2 Future work

This method may not eliminate the interference of other metal oxides such as ZnO. Therefore, how to separate other metal oxides by the analytical method has become the research aim of the future work. The detection limit of TiO₂ nanoparticles is 15 µg/mL. The concentration of TiO₂ in industrial wastewater (such as paper mill wastewater, textile wastewater, and olive mill wastewater) is several pg/mL.[121] The present method should be improved in the future work for these industrial applications.

References

- [1] Sabu Thomas, Chin Han Chan, Laly A. Pothan, et al. Carbon black reinforcement in natural rubber in micro a nano length. *Natural Rubber Materials*. Chapter 6. RSC Publishing. 2014.
- [2] Alessandro Tredicucci. Quantum dots: long life in zero dimensions. *Nature Materials*. 2009, 8: 775-776.
- [3] Askari Mohammad Bagher. Quantum dots applications. *Sensors & Transducers*. 2016, 198(3): 37-43.
- [4] Peter Reiss, Myriam Protiere, and Liang Li. Core/Shell semiconductor nanocrystals, Wiley inter science. 2009, 5(2): 154-168.
- [5] M. Cahay. Quantum confinement VI: nanostructured materials and devices proceedings of the international symposium. The Electrochemical Society. 2001.
- [6] Ephrem O. Chukwuocha, Michael C. Onyeaju, Taylor S. T. Harry. Theoretical studies on the effect of confinement on quantum dots using the Brus equation. *World Journal of Condensed Matter Physics*. 2012, 2: 96-100.
- [7] Alexander L. Efros, David J. Nesbitt. Origin and control of blinking in quantum dots. *Nature Nanotechnology*. 2016, 11: 661-671.
- [8] Philippe M. Fauchet. Light emission from Si quantum dots. *Materials today*. 2005, 1(8): 26-33.

-
- [9] Ou Chen, Jing Zhao, Vikash P. Chauhan, Jian Cui, Cliff Wong, et al. Compact high-quality CdSe–CdS core–shell nanocrystals with narrow emission linewidths and suppressed blinking. *Nature Materials*. 2013, 12: 445-451.
- [10] Vikram C.Sundar, Mouni Bawendi, Klavs F. Jensen, et al. Full color emission from II-VI semiconductor quantum dot–polymer composites. *Advanced materials*. 2000, 15(12): 1102-1105.
- [11] Jayasmita Jana, Mainak Ganguly and Tarasankar Pal. Enlightening surface plasmon resonance effect of metal nanoparticles for practical spectroscopic application. *Royal Society of Chemistry*. 2016, 6: 86174-86211.
- [12] Juan Zhou, Yong Yang, and Chun-yang Zhang. Toward biocompatible semiconductor quantum dots: from biosynthesis and bioconjugation to biomedical application. *Chem. Rev.* 2015, 115 (21): 11669-11717.
- [13] Matthew J. Allen, Vincent C. Tung, Richard B. Kaner. Honeycomb carbon: a review of graphene. *Chemical Reviews*. 2009, 10(1): 1023-1031.
- [14] Alexander G. Kvashnin, Leonid A. Chernozatonskii, Boris I. Yakobson, et al. Phase diagram of quasi-two-dimensional carbon-from graphene to diamond. *Nanoletters*. 2014, 14(2): 676-681.
- [15] Konstantin Novoselov, Andre Konstantin Geim, Sergey Morozov, et al. Electric field effect in atomically thin carbon films. *Science*. 2004, 306(5696): 666-669.
- [16] Charalampos Androulidakis, Emmanuel N. Koukaras, Otakar Frank, et al. Failure processes in embedded monolayer graphene under axial compression. *Scientific Reports*. 2014, 4, 5271-52718.

-
- [17] Matthew J. Allen, Vincent C. Tung and Richard B. Kaner. Honeycomb carbon: a review of graphene. *Chemical Reviews*. 2009, 10(1):1023-1031.
- [18] Konstantin Novoselov, Andre Konstantin Geim, Sergey Morozov, et al. Electric Field Effect in Atomically Thin Carbon Films. *Science*. 2014, 8, 306(5696): 666-669.
- [19] <http://www.azonano.com/article.aspx?ArticleID=4264>.
- [20] Adriano Ambrosi and Chun Kiang Chua. Electrochemistry of graphene and related materials. *Chem. Rev.* 2014, 114: 7150-7188.
- [21] Jackie D. Renteria, Denis L. Nika and Alexander A. Balandin. Graphene thermal properties: applications in thermal management and energy storage. *Appl. Sci.* 2014, 4: 525-547.
- [22] Herb Brody. Graphene. *Nature Outlook*. 2012, 483(7389): S29–S44.
- [23] Paolo Elvati, Elizabeth Baumeister and Angela Violi. Graphene quantum dots: effect of size, composition and curvature on their assembly. *RSC Adv.* 2017, 7: 17704-17710.
- [24] Charalampos Androulidakis, Emmanuel N. Koukaras, Otakar Frank, et al. Failure processes in embedded monolayer graphene under axial compression. *Scientific Reports*. 2014, 4: 5271-5278.
- [25] Sofia Paulo, Emilio Palomares and Eugenia Martinez-Ferrero. Graphene and carbon quantum dot-based materials in photovoltaic devices: from synthesis to applications. *Nanomaterials*. 2016, 6, 157: 1-20.

-
- [26] Yunhai Li, Huabing Shu and Shudong Wang. Electronic and optical properties of graphene quantum dots: the role of many-body effects. *J. Phys. Chem. C*. 2015, 119 (9): 4983-4989.
- [27] Xianxian Yao, Xingxing Niu, Kexin Ma, et al. Graphene quantum dots-capped magnetic mesoporous silica nanoparticles as a multifunctional platform for controlled drug delivery, magnetic hyperthermia , and photothermal therapy. *Advanced science news*. 2017, 10.1002/sml.201602225.
- [28] Zonglong Zhu, Jiani Ma, Zilong Wang, et al. Efficiency enhancement of perovskite solar cells through fast electron extraction: the role of graphene quantum dots. *J. Am. Chem. Soc.* 2014, 136 (10): 3760–3763.
- [29] Nrusimhan Lakshmi and Pankaj Tambe. EMI shielding effectiveness of graphene decorated with graphene quantum dots and silver nanoparticles reinforced PVDF nanocomposites. *Composite Interfaces*. 2017, 9(24): 861-882.
- [30] Dengyu Pan, Jinkai Jiao, Zhen Li, et al. Efficient separation of electron-hole pairs in graphene quantum dots by TiO₂ heterojunctions for dye degradation. *Chemistry& Engineering*. 2015, 3: 2405-2413.
- [31] Dengyu Pan, Jinkai Jiao, Zhen Li, et al. Efficient separation of electron–hole pairs in graphene quantum dots by TiO₂ heterojunctions for dye degradation. *Chemistry& Engineering*. 2015, 3: 2405-2413.
- [32] Fitri Aulia Permatasari, Akfany Hasdi Aimon, Ferry Iskandar, et al. Role of C–N configurations in the photoluminescence of graphene quantum dots synthesized by a hydrothermal route. *Sci. Rep.* 2016, 6: 21042.

-
- [33] Sofia Paulo, Emilio Palomares and Eugenia Martinez-Ferrero. Graphene and carbon quantum dot-based materials in photovoltaic devices: from synthesis to applications. *Nanomaterials*. 2016, 6(9): 157.
- [34] Yunhai Li, Huabing Shu, Shudong Wang, et al. Electronic and optical properties of graphene quantum dots: the role of many-body effects. *J. Phys. Chem. C*. 2015, 119(9): 4983-4989.
- [35] Youfu Wang and Aiguo Hu. Carbon quantum dots: synthesis, properties and applications. *J. Mater. Chem. C*. 2014, 2: 6921-6939.
- [36] Juan Peng, Wei Gao, Bipin Kumar Gupta, et al. Graphene quantum dots derived from carbon fibers. *Nano Letter*. 2012, 12: 844-849.
- [37] Boundless. Fluorescence and Phosphorescence. *Physics, Boundless*. Chapter 2. 2013.
- [38] Somes K. Das, Chi Man Luk, William E Martin, et al. Size and dopant dependent single particle fluorescence properties of graphene quantum dots. *J. Phys. Chem. C*. 2015, 119: 17988-17994.
- [39] Namasivayam Dhenadhyalan, King-Chuen Lin, Raghupathy Suresh. Unravelling the multiple emissive states in citric-acid-derived carbon dots. *J. Phys. Chem. C*. 2016, 120 (2): 1252-1261.
- [40] Liping Lin, Mingcong Rong, Feng Luo, et al. Recent advances in graphene quantum dots for sensing. *J. Materials Today*. 2013, 10(20): 433-442.

-
- [41] Shoujun Zhu, Yubin Song, Joy Wang, et al. Photoluminescence mechanism in graphene quantum dots: quantum confinement effect and surface/edge state. *Nano Today*. 2017, 4(13): 10-14.
- [42] Yan Li, Yang Zhao, Huhu Cheng, et al. Nitrogen-doped graphene quantum dots with oxygen-rich functional groups. *Chem. Soc.* 2012, 134(1): 15-18.
- [43] Jiabin. Qiao, Hua Jiang, Haiwen Liu, et al. Bound states in nanoscale graphene quantum dots in a continuous graphene sheet. *Physical Review B*. 2017, 2(95): 8-15.
- [44] Fangwei Zhang, Fei Liu, Chong Wang, et al. Effect of lateral size of graphene quantum dots on their properties and application. *Mater. Interfaces*. 2016, 8(3): 2104-2110.
- [45] Yan Li, Hui Liu, Xinqian Liu, et al. Free-radical-assisted rapid synthesis of graphene quantum dots and their oxidizability studies. *Langmuir*. 2016, 32(34): 8641-8649.
- [46] Hanjun Sun, Li Wu, Weili Wei, et al. Recent advances in graphene quantum dots for sensing. *J. materials today*. 2013.10(20): 433-442.
- [47] Juan Peng, Wei Gao, Bipin Kumar Gupta, et al. Graphene quantum dots derived from carbon fibers. *Nano Letter*. 2012, 12 (2): 844-849.
- [48] Daniela Iannazzo, Ida Ziccarellia and Alessandro Pistone. Graphene quantum dots: multifunctional nanoplatfroms for anticancer therapy. *J. Mater. Chem. B*. 2017, 10.1039/C7TB00747G.

-
- [49] Youfu Wang, Aiguo Hu. Carbon quantum dots: synthesis, properties and applications. *J. Mater. Chem. C*. 2014, 2: 6921-6939.
- [50] Weijie Shi, Hai Fan, Shiyun Ai, et al. Preparation of fluorescent graphene quantum dots from humic acid for bioimaging application. *New J. Chem.* 2015, 39: 7054-7059.
- [51] Somes K. Das, Chi Man Luk, William E. Martin, et al. Size and dopant dependent single particle fluorescence properties of graphene quantum dots. *J. Phys. Chem. C*. 2015, 119 (31): 17988-17994.
- [52] Xiaoming Li, Shengli Zhang, Sergei A. Kulinich, et al. Engineering surface states of carbon dots to achieve controllable luminescence for solid-luminescent composites and sensitive Be^{2+} detection. *Scientific Reports*. 2014, 2(18): 4976-4982.
- [53] Dengyu Pan, Zhang Jingchun, Zhen li, et al. Hydrothermal route for cutting graphene sheets into blue-luminescent graphene quantum dots. *Adv. Mater.* 2010(22): 734-738.
- [54] Eunhee Hwang, Hee Min Hwang, Yonghun Shin, et al. Chemically modulated graphene quantum dot for tuning the photoluminescence as novel sensory probe. *Sci Rep*. 2016, 6: 39448.
- [55] https://en.wikipedia.org/wiki/F%C3%B6rster_resonance_energy_transfer
- [56] Bastien D. Gomperts, Ijsbrand M. Kramer, Peter E.R. Tatham. Signal transduction. Elsevier, London. Chapter 4, 2009, 114-115.

-
- [57] Rüdiger Rudolf, Marco Mongillo, Rosario Rizzuto, et al. Looking forward to seeing calcium. *Nature Reviews*. 2003, 7(4), 579-586.
- [58] Deepika Bhatnagar, Vanish Kumar, Ashok Kumar, et al. Graphene quantum dots FRET based sensor for early detection of heart attack in human. *Biosensors and Bioelectronics*. 2016, 79: 495-499.
- [59] Juan Peng, Wei Gao, Bipin Kumar Gupta, et al. Graphene quantum dots derived from carbon fibers. *Nano Lett*. 2012, 12 (2): 844-849.
- [60] Dengyu Pan, Jingchun Zhang, Zheng Li, et al. Hydrothermal route for cutting graphene sheets into blue-luminescent graphene quantum dots. *Adv. Mater*. 2010, 22: 734-738.
- [61] Yongqiang Dong, Jingwei Shao, Congqiang Chen, et al. Blue luminescent graphene quantum dots and graphene oxide prepared by tuning the carbonization degree of citric acid. *Carbon*. 2012, 50, 12: 4738-4743.
- [62] Libin Tang, Rongbin Ji, Xiangke Cao, et al. Deep ultraviolet photoluminescence of water-soluble self-passivated graphene quantum dots. *ACS Nano*. 2012, 6 (6): 5102-5110.
- [63] Edward P.C. Lai, Graphene quantum dots for bioanalytical sensors and biochemical imaging applications. *J Res. Anal*. 2017, 2(3): 52-61.
- [64] Jingfei Liu, Lixia Qin, Shizhao Kang, et al. Gold nanoparticles/glycine derivatives/graphene quantum dots composite with tunable fluorescence and surface enhanced Raman scattering signals for cellular imaging. *Materials & Design*. 2017, 5 (123): 32-38.

-
- [65] Jian Ju, Ruizhong Zhang, Shuijian He, et al. Nitrogen-doped graphene quantum dots-based fluorescent probe for the sensitive turn-on detection of glutathione and its cellular imaging. *RSC Adv.* 2014, 4: 52583-52589.
- [66] Goki Eda, Yueyue Lin, Cecilia Mattevi, et al. Blue photoluminescence from chemically derived graphene oxide. *Adv. Mater.* 2010, 4 (22): 505-509.
- [67] Shoujun Zhu, Junhu Zhang, Chunyan Qiao, et al. Strongly green-photoluminescent graphene quantum dots for bioimaging applications. *Chem. Commun.* 2011, 47: 6858-6860.
- [68] Lishuang Fan, Yuwei Hu, Xiao Wang, et al. Fluorescence resonance energy transfer quenching at the surface of graphene quantum dots for ultrasensitive detection of TNT. *Talanta.* 2012, 15 (101): 192-197.
- [69] Yi Liu, Wweiquan Loh, Arundithi Ananthanarayanan, et al. Fluorescence quenching between unbonded graphene quantum dots and gold nanoparticles upon simple mixing. *RSC Advances.* 2014, 4, 35673.
- [70] Yuezhen He, Jian Sun, Dexiang Feng, et al. Multi-positively charged dendrimeric nanoparticles induced fluorescence quenching of graphene quantum dots for heparin and chondroitin sulfate detection. *Biosensors and Bioelectronics.* 2015, 74: 284-290.
- [71] Jianpo Zhang, Lihua Na, Yunxia Jiang, et al. Graphene quantum dots as a fluorescence-quenching probe for quantitative analysis of Ponceau 4R solution. *Analytical Methods.* 2016, 8(39): 7242-7246.

-
- [72] Jungki Ryu, Sook Hee Ku, Minah Lee, et al. Bone-like peptide/hydroxyapatite nanocomposites assembled with multi-level hierarchical structures. *Soft Matter*. 2011, 7, 7201-7206.
- [73] Rahila Batul, Tasnuva Tamanna, Abdul Khaliq, et al. Recent progress in the biomedical applications of polydopamine nanostructures. *Biomaterial Science* 2017, advanced article, DOI: 10.1039/C7BM00187H
- [74] Vincent Ball. Polydopamine films and particles with catalytic activity. *Catalysts* Today in press.
<http://www.sciencedirect.com/science/article/pii/S0920586117300317>
- [75] Yonghui Ding, Michael Floren and Wei Tan. Mussel-inspired polydopamine for bio-surface functionalization. *Biosurface and Biotribology*. 2016, 2(4): 121-136.
- [76] Zhi Li, Lejing Liu, Alejandro González, et al. Bioinspired polydopamine induced assembly of ultrafine Fe(OH)₃ nanoparticles on halloysite toward highly efficient fire retardancy of epoxy resin via an action of interfacial catalysis. *Polymer Chemistry*. 2017, 7 (26), DOI: 10.1039/C7PY00660H
- [77] Vincent Ball. Physicochemical perspective on polydopamine and poly(catecholamine) films for their applications in biomaterial coatings. *Biointerphases*. 2014, 9, 030801.
- [78] Lin Liu, Yong Chang, Jie Yu, et al. Two-in-one polydopamine nanospheres for fluorescent determination of beta-amyloid oligomers and inhibition of beta-amyloid aggregation. *Sensors and Actuators B: Chemical*. 2017, 251: 359-365.

-
- [79] Zhenqiang Wang, Jixi Zhang, Feng Chen, et al. Fluorescent miRNA analysis enhanced by mesopore effects of polydopamine nanoquenchers. *Analyst*, 2017, Advance Article <http://dx.doi.org/10.1039/C7AN00950J>.
- [80] Lina Ma, Fuyao Liu, Zhen Lei, et al. A novel upconversion@polydopamine core@shell nanoparticle based aptameric biosensor for biosensing and imaging of cytochrome c inside living cells. *Biosensors and Bioelectronics*. 2017, 87: 638-645.
- [81] Minmin Dong, Chunli Liu, Shuying Li, et al. Polymerizing dopamine onto Q-graphene scaffolds towards the fluorescent nanocomposites with high aqueous stability and enhanced fluorescence for the fluorescence analysis and imaging of copper ions. *Sensors and Actuators B: Chemical*. 2016, 232: 234-242.
- [82] Xiangjuan Kong, Shuang Wu, Tingting Chen, et al. MnO₂-induced synthesis of fluorescent polydopamine nanoparticles for reduced glutathione sensing in human whole blood. *Nanoscale*. 2016, 8(34): 15604-15610.
- [83] Dong Fan, Xiaoqing Zhu, Qingfeng Zhai, et al. Polydopamine nanotubes as an effective fluorescent quencher for highly sensitive and selective detection of biomolecules assisted with exonuclease III amplification. *Analytical Chemistry*. 2016, 88(18): 9158-9165.
- [84] Shuaohuang Weng, Dong Liang, Huazhang Qiu, Zongen Liu, et al. A unique turn-off fluorescent strategy for sensing dopamine based on formed polydopamine using graphene quantum dots as fluorescent probe. *Sensors and Actuators B: Chemical*. 2015, 221: 7-14.

-
- [85] Jiahui Lin, Chengju Yu, Yachun Yang, et al. Formation of fluorescent polydopamine dots from hydroxyl radical-induced degradation of polydopamine nanoparticles. *Physical Chemistry Chemical Physics*. 2015, 17(23): 15124-15130.
- [86] Chun Kit K. Choi, Jinming Li, Kkongchang Wei, et al. A gold@polydopamine core-shell nanoprobe for long-term intracellular detection of microRNAs in differentiating stem cells. *Journal of the American Chemical Society*. 2015, 137(23): 7337-7346.
- [87] Weibing Qiang, Weri Li, Xiaoqing Li, et al. Bioinspired polydopamine nanospheres: a superquencher for fluorescence sensing of biomolecules. *Chemical Science*. 2014, 5(8): 3018-3024.
- [88] Vincent Ball. Physicochemical perspective on polydopamine and poly(catecholamine) films for their applications in biomaterial coatings. *Biointerphases* 2014, 9: 030801.
- [89] Musharraf Miah, Zafar Iqbal, Edward P.C. Lai. A comparative CE-UV binding tests study between polydopamine-coated and polypyrrole-coated magnetic nanoparticles with endocrine disrupting compounds and pharmaceuticals in water. *CLEAN Soil Air Water*. 2014, 43(2): 173-181.
- [90] Zafar Iqbal, Edward P.C. Lai. Investigation of polydopamine growth on nanomaterials in water. *Journal of Nanomaterials and Molecular Nanotechnology*. 2013, 2(6): doi: 10.4172/2324-8777.1000127.

-
- [91] Zafar Iqbal, Edward P.C. Lai, T.J. Avis. Antimicrobial effect of polydopamine coating on *Escherichia coli*. *Journal of Materials Chemistry* 2012, 22: 21608–21612.
- [92] Sylwia Baluta, Karol Malecha, Dorota Zajac, et al. Dopamine sensing with fluorescence strategy based on low temperature co-fired ceramic technology modified with conducting polymers. *Sensors and Actuators B: Chemical* 2017, 7(12), DOI: 10.1016/j.snb.2017.06.073.
- [93] Lu Ding, Zhenwen Qin, Chunlan Xiang, G. Zhou. Novel fluorescent organic nanoparticles as a label-free biosensor for dopamine in serum. *Journal of Materials Chemistry B* 2017, 5, 2750–2756.
- [94] Zheqian Wang, Jixi Zhang, Feng Chen, et al. Fluorescent miRNA analysis enhanced by mesopore effects of polydopamine nanoquenchers. *Analyst*. 2017, June 13, DOI: 10.1039/C7AN00528H
- [95] Sung Hee Jooa, Dongye Zhao. Environmental dynamics of metal oxide nanoparticles in heterogeneous systems: A review. *Journal of Hazardous Materials*. 2017, 322: 29-47.
- [96] Berna Dalkiran, Pinar Esra Erden and Esma Kiliç. Amperometric biosensors based on carboxylated multiwalled carbon nanotubes-metal oxide nanoparticles-7, 7, 8, 8-tetracyanoquinodimethane composite for the determination of xanthine. *Talanta*. 2017, 167, 286-295.
- [97] Muthuchamy Maruthupandy, Yong Zuo, Jing-Shuai Chen, et al. Synthesis of metal oxide nanoparticles (CuO and ZnO NPs) via biological template and their optical sensor applications. *Applied Surface Science*. 2017, 397, 167-174.

-
- [98] Ye He, Fan Mo, Danlong Chen, et al. Capillary electrophoresis inductively coupled plasma mass spectrometry combined with metal tag for ultrasensitively determining trace saxitoxin in seafood. *Electrophoresis*. 2017, 38, 469-476.
- [99] Samar Alsudir and Edward P. C. Lai. Electrosteric stabilization of colloidal TiO₂ nanoparticles with DNA and polyethylene glycol for selective enhancement of UV detection sensitivity in capillary electrophoresis analysis. *Bioanal Chem*. 2017, 409: 1857-1868.
- [100] Samar Alsudir, Edward P.C. Lai. Selective detection of ZnO nanoparticles in aqueous suspension by capillary electrophoresis analysis using dithiothreitol and L-cysteine adsorbates. *Talanta*. 2017, 169: 115-122.
- [101] Ariel R. Donovan, Craig D. Adams, Yinfu Ma, et al. Detection of zinc oxide and cerium dioxide nanoparticles during drinking water treatment by rapid single particle ICP-MS methods. *Analytical and Bioanalytical Chemistry*. 2016, 408(19): 5137–5145.
- [102] Laurie Fréchette-Viens, Madjid Hadioui and Kevin J. Wilkinson. Practical limitations of single particle ICP-MS in the determination of nanoparticle size distributions and dissolution: case of rare earth oxides. *Talanta*. 2017, 163: 121-126.
- [103] Samar Alsudir and Edward P.C. Lai. Polymer coatings for sensitive analysis of colloidal silica nanoparticles in water. *Colloid and Polymer Science*. 2014, 292: 1289-1296. DOI: 10.1007/s00396-014-3184-1.

-
- [104] Samar Alsudir and Edward P.C. Lai. Enhancement of detection sensitivity for colloidal nanoparticles in capillary electrophoresis analysis. *Journal of Analytical & Bioanalytical Techniques*. 2015, 6(3): 1000242.
- [105] Samar Alsudir and E.P.C. Lai. Electrosteric stabilization of colloidal TiO₂ nanoparticles with DNA and polyethylene glycol for selective enhancement of UV detection sensitivity in capillary electrophoresis analysis. *Analytical and Bioanalytical Chemistry*. 2017, 409(7): 1857-1868.
- [106] Samar Alsudir and E.P.C. Lai. Selective detection of ZnO nanoparticles in aqueous suspension by capillary electrophoresis analysis using dithiothreitol and L-cysteine adsorbates. *Talanta*. 2017, 169: 115-122.
- [107] Xi Zhou, Peipei Ma, Anqi WangM et al. Dopamine fluorescent sensors based on polypyrrole/graphene quantum dots core/shell hybrids. *Biosensors and Bioelectronics*. 2015, 64: 404-410.
- [108] J.R.S. Brownson, M.I. Tejedor-Tejedor, M.A. Anderson. FTIR spectroscopy of alcohol and formate interactions with mesoporous TiO₂ surfaces. *J. Phys. Chem. B*. 2006, 110: 12494-12499.
- [109] Yongqiang Dong, Jingwei Shao, Congqiang Chen, et al. Blue luminescent graphene quantum dots and graphene oxide prepared by tuning the carbonization degree of citric acid. *Elsevier*. 2012, 12(50): 4738-4743.
- [110] Rolf Koole, Esther Groeneveld, Daniel Vanmaekelbergh, et al. Size effects on semiconductor nanoparticles. *Nanoparticles*, Springer-Verlag, Berlin. Chapter 2. 2014, 13-51.

-
- [111] Juan Peng, Wei Gao, Bipin Kumar Gupta, et al. Graphene quantum dots derived from carbon fibers. *Nano Letter*. 2012, 12 (2): 844-849.
- [112] Daniela Iannazzo, Ida Ziccarellia and Alessandro Pistone. Graphene quantum dots: multifunctional nanoplatfroms for anticancer therapy. *J. Mater. Chem. B*. 2017, 10.1039/C7TB00747G.
- [113] Rebecca A. Zangmeister, Todd A. Morris and Michael J. Tarlov. Characterization of polydopamine thin films deposited at short times by autoxidation of dopamine. *Langmuir*. 2013, 29(27): 8619-8628.
- [114] Andrea León, Patricia Reuquen, Carolina Garín, et al. FTIR and Raman Characterization of TiO₂ nanoparticles coated with polyethylene glycol as carrier for 2-methoxyestradiol. *Applied Sciences*. 2017, 7(1): 49.
- [115] Jürgen Liebscher, Radosław Mrówczyński, Holger A. Scheidt Liebscher, et al. Structure of Polydopamine: A Never-Ending Story? *Langmuir*. 2013, 29(33), 10539-10548.
- [116] Suk-Ho Choi. Unique properties of graphene quantum dots and their applications in photonic/electronic devices. *Journal of Physics D: Applied Physics*. 2017, 50(10): 103002.
- [117] Elizabeth Carraway, James N. Demas and Benjamin DeGraff. Luminescence quenching mechanism for microheterogeneous systems. *Anal. Chem*. 1991, 63 (4): 332-336.
- [118] Mustafa Arık, Neslihan Çelebi, Yavuz Onganer. Fluorescence quenching of fluorescein with molecular oxygen in solution. *Journal of Photochemistry and Photobiology A: Chemistry*. 2005, 170(2): 105-111.

-
- [119] Huai You, WangYue and SunboTang. Study on fluorescence property of dopamine and determination of dopamine by fluorimetry. *Talanta*. 2002, 57(5): 899-907.
- [120] W.G. Kreyling, U. Holzwarth, N. Haberl, et al. Quantitative biokinetics of titanium dioxide nanoparticles after intravenous injection in rats: Part 1. *Nanotoxicology*. 2017, 11(4): 434–442.
- [120] <https://www.mrl.ucsb.edu/tempo-facility/instruments/horiba-fluoromax-4-spectrometer>.
- [121] Yongbing Wang, Chia-swee Hong, and Fu Fang. Effect of solution matrix on TiO₂ photocatalytic degradation of 2-chlorobiphenyl. *Environmental Engineering Science*. 2009, 16(6): 433-440.