ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

SYNTHESIS OF YELLOW-EMISSIVE CARBON DOTS AND OBSERVATION OF THEIR INTERACTION WITH AFLATOXIN B1

M.Sc. THESIS

Özge ERGÜDER

Department of Nanoscience and Nanoengineering

Nanoscience and Nanoengineering Programme

NOVEMBER 2022

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

SYNTHESIS OF YELLOW-EMISSIVE CARBON DOTS AND OBSERVATION OF THEIR INTERACTION WITH AFLATOXIN B1

M.Sc. THESIS

Özge ERGÜDER
(513201002)

Department of Nanoscience and Nanoengineering

Nanoscience and Nanoengineering Programme

Thesis Advisor: Assoc. Prof. Dr Caner ÜNLÜ

NOVEMBER 2022

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

SARI IŞIMA YAPAN KARBON NOKTALARININ SENTEZİ VE AFLATOKSİN B1 İLE ETKİLEŞİMLERİNİN GÖZLEMLENMESİ

YÜKSEK LİSANS TEZİ

Özge ERGÜDER
(513201002)

Nanobilim ve Nanomühendislik Anabilim Dalı

Nanobilim ve Nanomühendislik Programı

Tez Danışmanı: Doç. Dr. Caner ÜNLÜ

KASIM 2022

Özge ERGÜDER, a **M.Sc.** student of **ITU Graduate School** student ID 513201002, successfully defended the thesis entitled **"SYNTHESIS OF YELLOW-EMISSIVE CARBON DOTS AND OBSERVATION OF THEIR INTERACTION WITH AFLATOXIN B1"**, which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Assoc. Prof. Dr. Caner ÜNLÜ** Istanbul Technical University	.....
Jury Members :	**Assist. Prof. Dr. Ahmet Emin ATİK** Acıbadem Mehmet Ali Aydınlar University	.....
	Assist. Prof. Dr. Onur ALPTÜRK Istanbul Technical University	.....

Date of Submission : 25 October 2022
Date of Defense : 2 November 2022

To all my future journeys,

FOREWORD

I would like to thank my thesis supervisor Assoc. Prof. Dr Caner ÜNLÜ, for accepting me as his student, for his endless patience, for not sparing his material and moral opportunities, and for always enlightening my way with his wisdom. After my teacher, the person I want to thank the most is my teammate Sultan, whom I always work with... Sultan also became my dear friend, with who I walked on the same path, who supported me constantly and made me focus on my studies, and who contributed a lot to my work. These two people made me realize that a good science adventurer must first be a good person with endless sharing, benevolence and all other good habits.

I would like to thank; To my teacher Ilgın NAR, teacher Nilgün and Türkan who did not spare their knowledge, ideas and help throughout my work and taught me about the use of devices; To Beedot Research Group members, all labmates whose knowledge and experience I benefited from during my education. I am so happy to have had great conversations, had lunches like a picnic and worked with these special people. I am feeling rich thanks to Melek, Nida, Ömer Faruk, Merve, Yağız and Sümeyye.

I also want to thank Dr Levent TRABZON and the MEM-TEK lab group, I used their equipment and chemicals many times with feeling like a real part of the research group. I thank you to Dr Sedat NIZAMOĞLU and his student Güncel Eren, who gave us their guidance and opportunity to use their fluorescence lifetime spectrophotometer; to Dr Ahmet GÜL for UV-vis, and; Dr Bünyamin KARAGÖZ for support and the use of the fluorescence spectrometer.

And, I have appreciated my family; my dad, mom, brother and Oğuz who have been by my side all my life, who have always supported me financially and morally, and who have always believed in and trusted me; My sincere thanks to everyone who contributed to this thesis.

October 2022

Özge ERGÜDER
(Bioengineer)

TABLE OF CONTENTS

Page

ABBREVIATIONS

Abs **:** Absorbance
AF **:** Aflatoxin
AFB1 **:** Aflatoxin B1
ATR **:** Attenuated Total Reflection
CB **:** Conduction Band
CD **:** Carbon Dot
CQD **:** Carbon Quantum Dot
DNA : Deoxyribonucleic acid
E **:** Energy Efficiency Transfer
Ems **:** Emission
mL **:** Milli Liter
µm **:** Mikro Meter
FRET **:** Förster Resonance Energy Transfer
FTIR **:** Fourier Transform Infrared Spectroscopy
FWHM **:** Full Width at Half Maximum
IR **:** Infrared
LED **:** Light Emitting Diode
MHz **:** Mega Hertz
MW **:** M Walt
NM **:** Nanometer
NS **:** Nanosecond
PD **:** Phenylene diamine
PL **:** Photoluminescence
PLE **:** Photoluminescence Excitation
RS **:** Raman Spectroscopy
QDs **:** Quantum Dots
QY **:** Quantum Yield
UV **:** Ultraviolet
VB **:** Valance Band

SYMBOLS

Ag	**:** Silver
Al	**:** Aluminium
Cu	**:** Copper
Ga	**:** Gallium
In	**:** Indium
S	: Sulphur
Se	**:** Selenium
Te	**:** Tellurium

LIST OF TABLES

Page

LIST OF FIGURES

Page

SYNTHESIS OF YELLOW-EMISSIVE CARBON DOTS AND OBSERVATION OF THEIR INTERACTION WITH AFLATOXIN B1

SUMMARY

The field of nanoscience focuses on topics between 1 and 100 nm in size, while the field of nanotechnology is primarily concerned with the development of useful tools and equipment for this scale. Although the term "nanotechnology" was coined during the last decade, researchers have been hard at work in this field since the 1950s. It is anticipated that this new technology, whose dimensions are measured in nanometers, would significantly advance the state of the art. Nanotechnology has fascinating potential uses in almost every area of study, from physics and materials science to biology and biotechnology to computer engineering and electronics, and even medicine. The word "nanobiotechnology" is an obvious portmanteau of "nanotechnology" and "biology," as it describes the use of nanotools and nanodevices to interact with, monitor, and modify biological functions at the cellular and molecular levels.

Electron transport in nanoscale crystals known as quantum dots (QDs) is an engineering feat. These semiconducting nanoparticles can produce a rainbow of colors when exposed to ultraviolet light. QDs are semiconductor nanoparticles, first postulated in the 1970s and first realized in the early 1980s. The phrase "dots" refers to nanocrystals, while the term "quantum" refers to the smallest and most discrete unit of any physical attribute. QDs have the same arrangement of atoms as in the bulk matter, unlike bulk and nanocrystal quantum dots, therefore much more surface atoms are shown on the surface by virtue of three-dimensional truncation. Sizes of QDs are typically between 2 and 10 nm. Optical characteristics and fluorescence are produced by the semiconductor's basic material. In particular, the core material displays impacts of toxicology, photobleaching, and blinking. Because of their unique physicochemical properties, QDs can be used in a wide range of industries and technologies, from medicine and medicine delivery to electronics and solar power.

The two main forms of QD manufacturing are core (bare QDs) and core/shell architectures. The shell is primarily composed of inorganic substance in order to stabilize the core, which is primarily derived from semiconductors. The semiconductor core of QDs may or may not be surrounded by a semiconductor shell. QDs range in size from 2-10 nm. The core material of the semiconductor is responsible for optical properties and fluorescence. Specifically, core material exhibits blinking, photobleaching, and toxicological effects. In core/shell arrangement, the core is passivated by the shell (which often has a wider band-gap), which improves optical quality and prevents leaching. Therefore, core/shell structures are preferable to bare QDs for biological purpose. For biological studies, it is important to modify the QDs to make them water-dispersible. Therefore, the surface of QDs can be modified with various bifunctional surface ligands or caps to enhance their solubility in aqueous media. The modification of QDs can improve their quantum yield (QY) and stability, reduce surface oxidation, and limit the release of hazardous ions.

Understanding the composition, shape, crystalline structure, bandgap energy, and surface ligand nature of QDs is crucial for exploiting their optoelectric and physicochemical properties. Characterization techniques for these nanoparticles mostly fall into two categories: optical characterization and structural characterization. In order to get optical information on QDs, such as their concentration, size, and photoluminescence quantum, the UV-Vis and fluorescence spectroscopy methods are frequently employed. Structure investigation of QDs by X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) reveals information about the semiconductor nanocrystals' size, shape, crystal structure, and chemical composition. Some laboratories also employ Raman spectroscopy, which provides a fast and precise method for monitoring atomic vibrational changes. Although there are more than 20 different types of aflatoxins, Aflatoxin B1 (AFB1) is widely considered to be the most dangerous because it may contaminate a wide variety of crops. One biomolecule, AFB1, can emit a fluorescent signal.

In 2004, researchers developed carbon quantum dots (CQDs), also known as carbon dots (CDs), a class of multifunctional fluorescent carbon-based nanomaterials. Water dispersibility, low toxicity, high photoluminescence emission, cell membrane permeability, biocompatibility, and inexpensive manufacture are only a few of the many outstanding properties of carbon quantum dots. These are put to use in analytical imaging, drug delivery, light-emitting devices, and the field of biology. Cell-specific targeting, *in-vivo* imaging, and live cell labeling are all made possible through the microwave-assisted synthesis of carbon quantum dots. CDs are promising for *in-vivo* and *in-vitro* imaging research, but their fluorescence is most intense between 430 nm and 530 nm, which overlaps with the autofluorescence region of many eukaryotic cells. The development of high-quantum-yield yellow-red emissive CDs is thus a topic of interest. In this research, conventional yellow-emissive CDs were improved in two ways that increased their quantum yield. 1) Using simple, fast, and cheap microwave-assisted manufacturing procedures, the surface of conventional yellow-emitting CDs was passivated with a biomolecule, urea. 2) Yellow-emitting CDs were powered by the fluorescent biomolecule aflatoxin B1. First approach achieves 51% quantum yield for CDs. Energy was transferred from AFB1 to CDs efficiently (over 40%) in the second approach. Our results demonstrated that simple-rapid microwave aided synthesis methods can be used to produce highly luminous yellow emissive CDs, which are promising candidates for sensing Aflatoxin B1. AFB1 was also found to be an emission booster for CDs, further supporting our findings. This thesis shows that traditional yellow emissive carbon dots can have their quantum yield improved by passivating their surfaces with urea using a microwave-assisted manufacturing process. Carbon dots' steady-state emission properties were unaffected by surface passivation, but the quantum yield and average fluorescence lifetime were greatly enhanced. After coming into contact with AFB1, the yellow emitted carbon dots' emission intensity was significantly increased. This study, found that AFB1 acted as an energy donor for carbon dots, resulting in a significant increase in fluorescence lifespan and quantum yield, which they measured. These results not only proved that yellow emissive carbon dots have a bright future as a chemosensor for AFB1, but they also hinted that AFB1 might be used as a suitable emission-intensity-booster for yellow emissive carbon dots in biomedical applications like *in-vivo* cell imaging.

SARI IŞIMA YAPAN KARBON NOKTALARININ SENTEZİ VE AFLATOKSİN B1 İLE ETKİLEŞİMLERİNİN GÖZLEMLENMESİ

ÖZET

Nanobilim alanı 1 ile 100 nm arasındaki konulara odaklanırken, nanoteknoloji alanı öncelikle bu ölçek için faydalı araç ve gereçlerin geliştirilmesiyle ilgilenir. "Nanoteknoloji" terimi son on yılda ortaya çıkmış olsa da, araştırmacılar 1950'lerden beri bu alanda çok çalışmaktalardır. Boyutları nanometre cinsinden ölçülen bu yeni teknolojinin son teknolojiyi önemLi ölçüde ilerleteceği öngörülmektedir. Nanoteknoloji, fizik ve malzeme biliminden biyoloji ve biyoteknolojiye, bilgisayar mühendisliği ve elektroniğe ve hatta tıbba kadar hemen hemen her çalışma alanında büyüleyici potansiyel kullanımLara sahiptir. "Nanobiyoteknoloji" kelimesi, hücresel ve moleküler seviyelerde biyolojik işlevlerle etkileşime girmek, bunları izlemek ve değiştirmek için nanoaraçların ve nanocihazların kullanımını tanımLadığı için "nanoteknoloji" ve "biyoloji"nin karışımıdır.

Kuantum noktaları, (QD'ler) olarak bilinen nano ölçekli kristallerde elektron taşınması bir mühendislik başarısıdır. Bu yarı iletken nanopartiküller, ultraviyole ışığa maruz kaldıklarında bir gökkuşağı rengi spektrumunda çeşitli dalga boylarında ışıma yapacak şekilde üretebilir. Kuantum noktaları, ilk olarak 1970'lerde öne sürülen ve ilk olarak 1980'lerin başında gerçekleştirilmiş yarı iletken nanoparçacıklardır. "Noktalar" ifadesi nanokristallere atıfta bulunurken, "kuantum" terimi herhangi bir fiziksel özelliğin en küçük ve en ayrı birimine atıfta bulunur. Kuantum noktaları (QD'ler), buk ve nanokristal kuantum noktalarından farklı olarak, yığın maddedekiyle aynı atom düzenine sahiptir, bu nedenle, üç boyutlu kesme sayesinde yüzeyde çok daha fazla yüzey atomu gösterilir. QD'lerin boyutları tipik olarak 2 ile 10 nm arasındadır. Optik özellikler ve floresan, yarı iletkenin temel malzemesi tarafından üretilir. Özellikle çekirdek malzeme toksikoloji, ışıkla ağartma ve yanıp sönmenin etkilerini gösterir. Eşsiz fizikokimyasal özellikleri nedeniyle QD'ler, tıp ve ilaç dağıtımından elektronik ve güneş enerjisine kadar çok çeşitli endüstri ve teknolojilerde kullanılabilir.

QD üretiminin iki ana biçimi, çekirdek (çekirdek QD'ler) ve çekirdek/kabuk yapılarıdır. Kabuk, esas olarak yarı iletkenlerden türetilen çekirdeği stabilize etmek için öncelikle inorganik maddelerden oluşur. QD'lerin yarı iletken çekirdeği, bir yarı iletken kabuk ile çevrili olabilir veya olmayabilir. QD'lerin boyutu 2-10 nm arasında değişir. Bir yarı iletkenin, çekirdek yapısı optik özelliklerden ve floresanstan sorumludur. Spesifik olarak, çekirdek yapı yanıp sönme, fotoağartma ve toksikolojik etmenlerden sorumludur. Çekirdek/kabuk düzenlemesinde, çekirdek, optik kaliteyi artıran ve sızıntıyı önleyen kabuk (genellikle daha geniş bir bant aralığına sahiptir) tarafından pasifleştirilir. Bu nedenle, biyolojik amaçlar için çekirdek/kabuk yapıları çıplak QD'lere tercih edilir. Biyolojik çalışmalar için, QD'leri suda dağılabilir hale getirmek için değiştirmek önemlidir. Bu nedenle, QD'lerin yüzeyi, sulu ortamlarda çözünürlüklerini arttırmak için çeşitli iki işlevli yüzey ligandları veya kapakları ile modifiye edilebilir. QD'lerin modifikasyonu, kuantum verimini (QY) ve stabilitesini

iyileştirebilir, yüzey oksidasyonunu azaltabilir ve tehlikeli iyonların salınımını sınırlayabilir.

QD'lerin bileşimini, şeklini, kristal yapısını, bant aralığı enerjisini ve yüzey ligand yapısını anlamak, optoelektrik ve fizikokimyasal özelliklerinden yararlanmak için çok önemLidir. Bu nanopartiküller için karakterizasyon teknikleri çoğunlukla; optik karakterizasyon ve yapısal karakterizasyon olmak üzere iki gruba ayrılır. Konsantrasyonları, boyutları ve fotolüminesans kuantumLarı gibi QD'ler hakkında optik bilgi elde etmek için UV-Vis ve floresan spektroskopi yöntemLeri sıklıkla kullanılır. QD'lerin X-ışını kırınımı, X-ışını fotoelektron spektroskopisi, transmisyon elektron mikroskobu ve taramalı elektron mikroskobu ile yapı araştırması, yarı iletken nanokristallerin boyutu, şekli, kristal yapısı ve kimyasal bileşimi hakkında bilgi verir. Bazı laboratuvarlar, atomik titreşim değişikliklerini izlemek için hızlı ve kesin bir yöntem sağlayan Raman spektroskopisini de kullanır. 20'den fazla farklı aflatoksin türü olmasına rağmen, Aflatoksin B1 (AFB1), çok çeşitli mahsulleri kontamine edebileceği için yaygın olarak en tehlikeli olarak kabul edilir. Bir biyomolekül olan AFB1, bir floresan sinyali yayabilir. 2004 yılında araştırmacılar, çok işlevli floresan karbon bazlı nanomalzemelerin bir sınıfı olan karbon noktaları (CD'ler) olarak da bilinen karbon kuantum noktaları (CQD'ler) geliştirdiler. Suda dağılabilirlik, düşük toksisite, yüksek fotolüminesans emisyonu, hücre zarı geçirgenliği, biyouyumLuluk ve ucuz üretim, karbon kuantum noktalarının birçok olağanüstü özelliğinden sadece birkaçıdır. Bunlar analitik görüntülemede, ilaç dağıtımında, ışık yayan cihazlarda ve biyoloji alanında kullanılmaya başlandı. Hücreye özgü hedefleme, in-vivo görüntüleme ve canlı hücre etiketlemenin tümü, karbon kuantum noktalarının mikrodalga destekli sentezi yoluyla mümkün kılınır. CD'ler in-vivo ve in-vitro görüntüleme araştırmaları için umut vericidir, ancak floresansları en yoğun 430 nm ile 530 nm arasındadır ve bu, birçok ökaryotik hücrenin otofloresan bölgesi ile örtüşür. Bu nedenle, yüksek kuantum verimLi sarı-kırmızı yayıcı CD'lerin geliştirilmesi ilgi çeken bir konudur. Bu araştırmada, geleneksel sarı yayan CD'ler, kuantum verimLerini artıran iki şekilde geliştirildi. 1) Basit, hızlı ve ucuz mikrodalga destekli üretim prosedürleri kullanılarak, geleneksel sarı yayan CD'lerin yüzeyi bir biyomolekül, üre ile pasifleştirildi. 2) Sarı yayan CD'ler, floresan biyomolekül aflatoksin B1 tarafından desteklendi. İlk yaklaşım, CD'ler için %51 kuantum verimine ulaşır. İkinci yaklaşımda enerji AFB1'den CD'lere verimLi bir şekilde (%40'ın üzerinde) aktarılmıştır. Sonuçlarımız, basit-hızlı mikrodalga destekli sentez yöntemLerinin, aflatoksin B1'i algılamak için umut verici adaylar olan oldukça parlak sarı yayıcı CD'ler üretmek için kullanılabileceğini göstermiştir. AFB1'in ayrıca CD'ler için bir emisyon güçlendirici olduğu bulundu ve bu da bulgularımızı daha da desteklemektedir.

Bu tez, geleneksel sarı emisyonlu karbon noktalarının, mikrodalga destekli bir üretim süreci kullanarak yüzeylerini üre ile pasifleştirerek kuantum verimLerinin iyileştirilebileceğini göstermektedir. Karbon noktalarının kararlı durum emisyon özellikleri, yüzey pasivasyonundan etkilenmedi, ancak kuantum verimLeri ve ortalama floresan ömürleri büyük ölçüde geliştirildi. AFB1 ile etkileştikten sonra, yayılan sarı karbon noktalarının emisyon yoğunluğu önemLi ölçüde arttı. AFB1'in karbon noktaları için bir enerji vericisi olarak hareket ettiğini ve bunun, ölçtükleri floresan ömründe ve kuantum veriminde önemLi bir artışa neden olduğunu buldular. Bu sonuçlar, yalnızca sarı emisyonlu karbon noktalarının AFB1 için bir kemosensör olarak parlak bir geleceğe sahip olduğunu kanıtlamakla kalmadı, aynı zamanda AFB1'in *in-vivo* hücre gibi biyomedikal uygulamalarda, sarı ışıma yapan karbon

noktaları için uygun bir emisyon yoğunluğu artırıcı molekül olarak kullanılabileceğini gösterdi.

1. INTRODUCTION

1.1 Nanoscience & Nanotechnology

Nanoscience and nanotechnology comprise observe and manipulate matter in dimensions below 100 nm. As a prefix, ‘nano’ stems from the Greek prefix ‘dwarf’, a convenient word to indicate diminutiveness. To discriminate between nanoscience and nanotechnology, someone should focus on ‘science’ and ‘technology’ words. Nanoscience deals with matters on the scales of nanometers in 1-100 nm, whereas nanotechnology centers on applications and devices of this scientific area. While nanotechnology is a concept that has been introduced in the last ten years, intensive research has been carried out in the world since the 1950s. It is predicted that this new technology, which has dimensions measured in nanometers, will take existing technological achievements to a much higher level. Although current technologies deal with the known physical properties of materials, new properties called the quantum effect, which changes with the size of the material, emerge when nanoscales are approached [1]. In 1925, Richard Zsigmondy, who won Nobel Prize in the chemistry field, discovered the ‘nanometer’ term to explain the characterization of gold particle sizes using a microscope. After Zsigmondy, in 1965, another genius Richard Feynman won Nobel Prize in physics. After four years, Richard Feynman gave a presentation a lecture titled ‘There is Plenty of Room at the Bottom’. He introduced the concept of manipulating matter at the atomic level whic was motivated by the biological systems since majority of the operations in bodies take place at the nanoscale , which provides him to be remembered as the father of modern nanotechnology. Then, over a decade later, Norio Taniguchi first used ‘nanotechnology’ term to define semiconductor processes that occurred on the order of a nanometer [2].

There are intriguing applications of nanotechnology in practically every branch of research, including physics, materials science, biochemistry, biotechnology, computer engineering, electronics, and even human health [3]. The term nanobiotechnology is an obvious combination of nanotechnology with biology, enabling the utilization of nanotools and nanodevices to interact with, detection and modification of biological

operations at the cellular and molecular level [4]. As comparison examples, a single human hair is 60,000 nm in thickness, the DNA double helix has a radius of 1 nm, and the nuclear pores being 9 nm prevent substances of more than this size from entering the nucleus [1]. The Figure 1.1 represents comparisons of sizes of some nanomaterials and bionanomaterials.

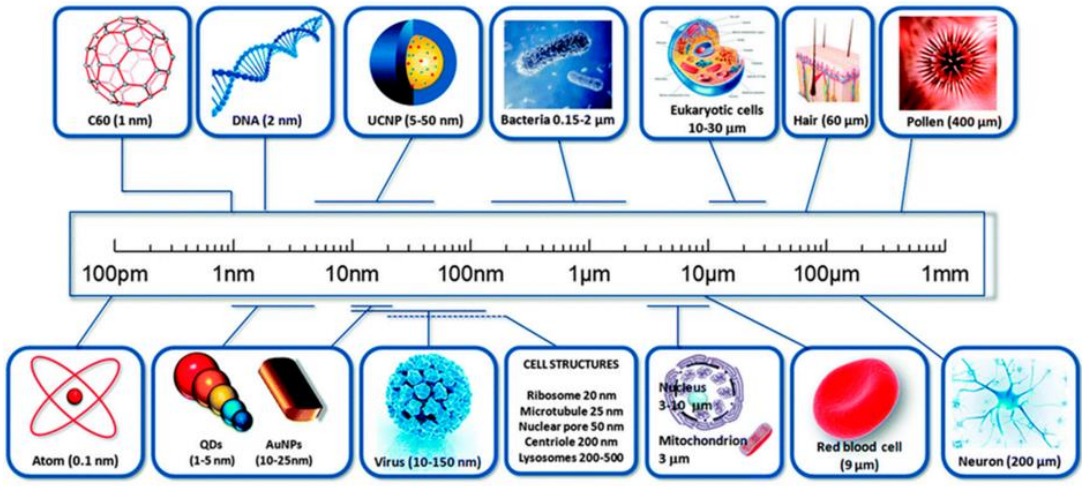

Figure 1.1: The comparison of sizes of nanomaterial and bionanomaterials [1]**.**

1.2 Quantum Dots

The term ‘quantum’ refers diminutive and separated unit of any physical property and ‘dots’ implies nanocrystals. Quantum dots are semiconducting nanoparticles or nanocrystals having a diameter between 2 and 10 nm (10–50 atoms) [2]. In technical terminology, QDs are defined as ‘small crystals consisting of a different number of electron groups that well-defined and separate quantum states and possess electronic features between bulk and separate main particle’. When we compare with buk and nanocrystal quantum dots, QDs possess the same arrangement of atoms as in the bulk matter, hence many more surface atoms are show on the surface by the virtue of three dimensional truncation. [3].

1.2.1 Historical background of quantum dots

Alexei Ekimov and Alexander Efros, colleagues works at the S. I. Vavilov State Optical Institute and A. F. Ioffe Institute in Russia, began researching and formulating ideas to explain the observed features of semiconductor-doped glasses about four decades ago [4]. Louis Brus at Bell Laboratories in Murray Hill, New Jersey, was studying semiconductor particles in liquid colloids simultaneously, but half a world away. These two lines of inquiry finally led to the independent development of

nanocrystals and theoretical explanation of their size-dependent optical features by two separate groups [5]. Next, in 1993, Weller provided the first description of the quantum confinement phenomena of the aqueous semiconductor quantum particle [6]. The same year, Murray and associates created a brand-new, repeatable technique for creating high-quality, nanocrystals which have narrow size distribution [7]. In 1998, QDs were originally introduced to the field of biological imaging as fluorophores with unique features that set them apart from conventional organic dyes. Their potential as diagnostic and therapeutic tools in nanobiotechnology and nanomedicine has been intensively studied since then, and over past two decades, extensive research has been done on their photophysical properties. The evolution of quantum dots from 1981 to 2017 is illustrated in Figure 1.2. The concept of bio-receptor immobilization is a new advancement, and the issue of predicting toxicity through the usage of QDs was introduced in 2010. In 2017, QDs were applied to medication delivery and tissue engineering for the first time. Additionally, the usage of QDs for imaging, diagnostics, and sensing was investigated [2].

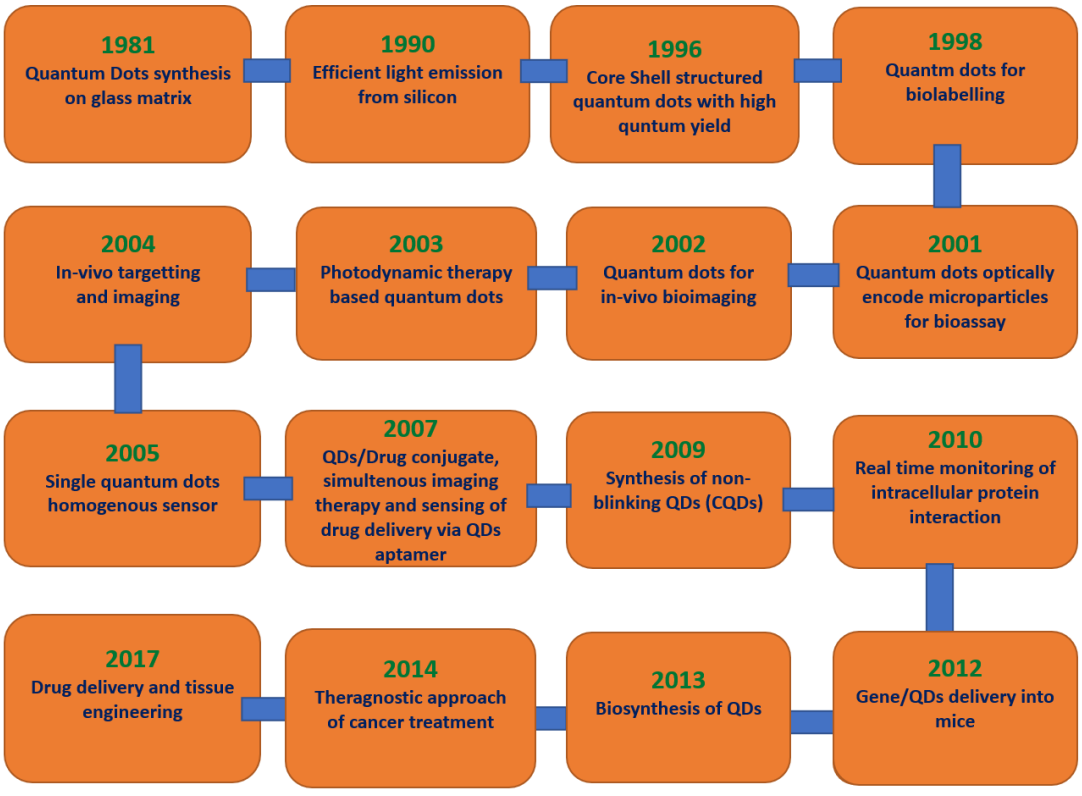

Figure 1.2: Chronological development of quantum dots, adapted from [2].

1.2.2 Quantum confinement effect

Based on electron conductivity, solid state physics classifies materials into three well-known groups: semiconductors, insulators, and conductors (metals). Quantum dots are a type of semiconductor. The energy level differential between the valance and conduction bands determines the conductivity of solid materials. Conduction band (CB) is the lowest electronic energy level that is not occupied by electrons, whereas valance band (VB) is the highest electronic energy level that electrons can occupy at room temperature. A positively charged hole forms in the valance band when an electron in the valance band reaches the conduction band after gaining energy through thermal or photon absorption. The amount of energy that an electron must obtain to reach the conduction band is determined by the band gap energy, which is the energy difference between the valance and conduction bands. The energy levels of electrons are "continuous" in semiconductor material in bulk form, but they become discrete in nanoforms due to quantum confinement [8]. Quantum confinement principles govern the emission and absorption spectra that correspond to the energy bandgap of the QDs, which is the energy needed to excite the electrons from the electronic band to higher energy levels. This excitation produces an electron-hole pair on its own, allowing it to emit fluorescent photons as a source of energy. The bandgap of QDs can be precisely adjusted by changing their sizes, and they can also be thought of as artificial atoms that can generate discrete energy levels. With a reduction in the size of the quantum dot particle and related wavelengths, the energy bandgap rises in particular. When QDs are produced at smaller sizes, electron hole pairs are squeezed more tightly, which results in higher energy levels and shorter wavelengths (blue), whereas larger QDs emit red light. The relationship between energy and light wavelength is inverse [3].

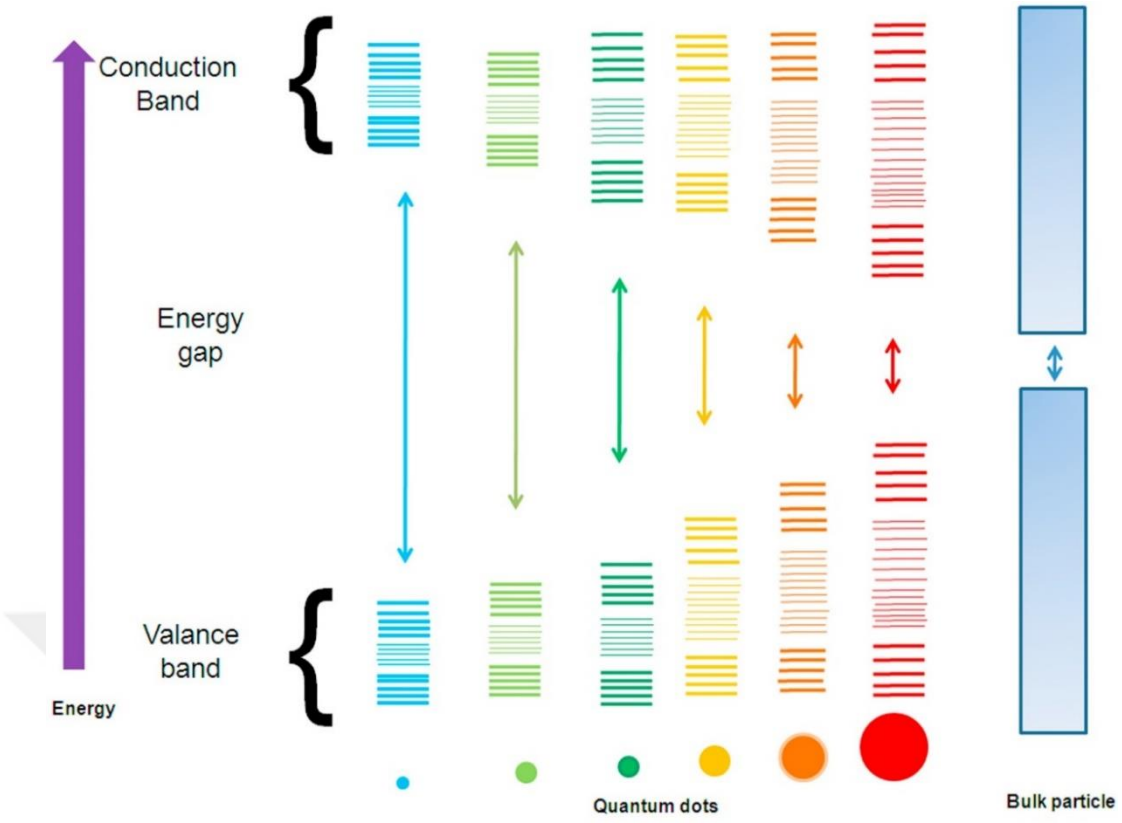

Figure 1.3: Energy gap comparison of various size of quantum dots and semiconductor bulk particle [3].

1.2.3 Structure & features of quantum dots

The two main forms of QD manufacturing are core (bare QDs) and core/shell architectures. The shell is primarily composed of inorganic substance in order to stabilize the core, which is primarily derived from semiconductors. The semiconductor core of QDs may or may not be surrounded by a semiconductor shell. QDs range in size from 2-10 nm. The core material of the semiconductor is responsible for optical properties and fluorescence. Specifically, core material exhibits blinking, photobleaching, and toxicological effects [2]. In core/shell arrangement, the core is passivated by the shell (which often has a wider band-gap), which improves optical quality and prevents leaching. Therefore, core/shell structures are preferable to bare QDs for biological purposes [9]. For biological studies, it is important to modify the QDs to make them water-dispersible. Therefore, the surface of QDs can be modified with various bifunctional surface ligands or caps to enhance their solubility in aqueous media [10]. The modification of QDs can improve their quantum yield (QY) and stability, reduce surface oxidation, and limit the release of hazardous ions [11]. Surface modification of QDs can be categorized into two categories: ligand exchange and

encapsulation. The ligand exchange method involves exchanging hydrophobic surfactant molecules with bifunctional structures. One of the encapsulation processes of QDs is silanization, the formation of a silica shell around the surface of the QDs. Coating the surfaces of QDs with phospholipid micelles, microspheres, amphiphilic polymers, etc. is another method for encapsulating QDs [10]. Capping chemicals and ligands play a crucial role in QD applications. How QDs are ultimately put to use is very reliant on the specifics of the capping agent that is created during the various synthesis steps. Common ligands utilized in the production of quantum dots include alcohols, primary amines, carboxylic acids, thiols, and long chain organophosphorates (QDs). These ligands allow for bioconjugation to occur between peptides, carbohydrates, DNA fragments, viruses, and natural products through hydrophobic interactions or electrostatic and covalent coupling. Ligands are the primary determinants of colloidal stability, solubility, particle size distribution, and particle shape; they prevent uncontrollable expansion and aggregation [3].

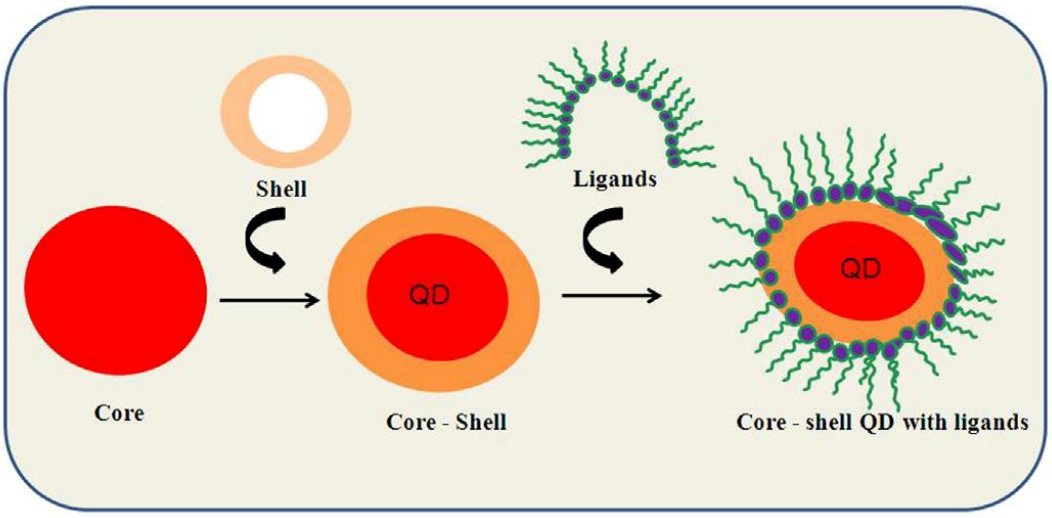

Figure 1.4: The comparison of sizes of nanomaterial and bionanomaterials [3].

QDs have unique optical properties compared to those of bulk solids because electron hole pairs (excitons) are confined to the grain boundary of the nanocrystals. High fluorescent quantum yields, wide absorption extinction coefficients, robust to the photo bleach effect, reduced fluorescence periodicity, size and composition adjustable light emission, and a significant Stokes shift are only a few of the photophysical characteristics [3]. Due to their special physico-chemical characteristics, QDs are well suited for a variety of applications, including imaging, medication administration, gene

therapy, displays, electronics, and solar energy. The Figure 1.5 indicates where quantum dots are used in detailed.

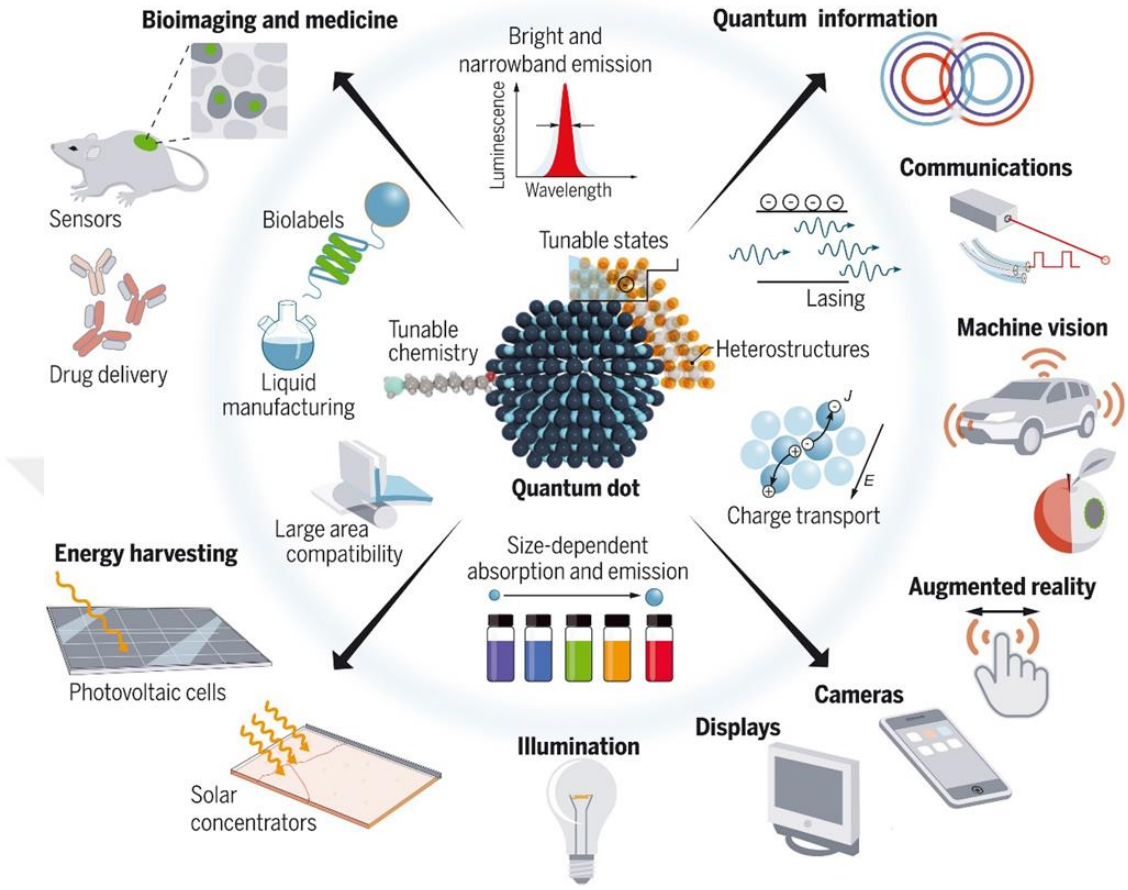

Figure 1.5 Illustration of application areas of quantum dot technology [12].

1.2.4 Types of quantum dots

QDs can be categorized into three basic categories: (a) according to their composition/structure; (b) according to size; and (c) according to the material utilized in their creation. According to the structure, QDs are categorized as core-based QDs, core-shell QDs, and alloyed quantum dots. Another form of classification is made by according to size as larger QDs and smaller QDs. In applications like photovoltaics and LEDs, larger QDs with a diameter of 5-6 nm emit orange or red light at a longer wavelength, whereas smaller QDs with a diameter of around 2-3 nm emit blue or green light at shorter wavelengths. [2]. Lastly but most importantly, QDs are primally categorized based on which material they are occurred. They are divided into two groups: including heavy metals and QDs excluding heavy metals. The presence of heavy-metal cations, such as cadmium, lead, and mercury, in conventional colloidal QDs has attracted attention. Typically, they are mixtures of transition metals and

metalloid elements, such as those from groups III-V, II-VI, and IV-VI. Notably, cadmium-based QDs are the most widely used type in quantum dot technology because to their durability, high fluorescence yield, superior sensitivity, and simple technique of synthesis. Numerous investigations have demonstrated, however, that these Cd-containing QDs fail in undesired toxicity concerns [13]. Carbon-based QDs, silicon QDs, and heavy metal-free ternary group I-III-VI QDs, (Group I (Cu, Ag), Group III (Al, Ga, In, Tl) and Group VI (S, Se, Te) elements) are heavy metal free quantum dots [14].

1.2.5 Carbon quantum dots

Carbon quantum dots (CQDs) are also called carbon dots (CDs) are versatile fluorescent carbon-based nanomaterials, was first discovered in 2004. Carbon quantum dots have brilliant features such as water-dispersibility, low toxicity, strong photoluminescence emission, cell membrane permeability, biocompatibility, cheaper production, etc. [15]. These are utilized in biological imaging, light-emitting devices, drug delivery, and analytical imaging. The microwave-assisted production of carbon quantum dots is performed to cell-specific targeting, *in-vivo* imaging, and live cell labeling [2]. Although conventional quantum dots, CDs are produced from nonrenewable precursors such as coal and petroleum coke, eco-friendly carbon-based QDs are now produced from renewable biomass wastes (walnut shell, coffee grounds, fennel seed, watermelon and orange peel). Numerous studies have demonstrated that carbon-based quantum dots are less cytotoxic than ordinary QD [16], [17]. CDs emit fluorescence in the blue-green wavelength range (430-530 nm) with the highest quantum yield; as a result, their utility in *in-vivo* imaging is limited since this wavelength range overlaps with the autofluorescence of the majority of eukaryotic cells (420-520 nm). Thus, the development of yellow-red emitting CDs (emission wavelength greater than 550 nm) with high QYs is of vital importance and is intensively researched. In general, 1,2-phenylenediamine (1,2-PD) and its isomers were employed as carbon precursors for the production of yellow-red emissive CDs. The hydrothermal synthesis method and the microwave aided synthesis method are often employed to create CDs by utilizing PD as a carbon precursor [18].

1.2.6 Synthesis of quantum dots

A key component of nanotechnology is the creation of nanoparticles with regulated size and shape. Numerous methods have been used to create semiconducting nanocrystals that have a wide range of properties, size and shapes since the discovery of quantum dots (QDs). QDs have often been produced using top-down methods like epitaxial growth and nanoscale patterning methods (lithography-based technology). The central point behind the top-down strategy is to use etching, slicing, or cutting to diminish materials from bulk to nanoscale. On the other hand, bottom-up techniques concentrate on the self-assembly of nanocrystal particles using colloidal chemistry [19], [20]. The following colloidal-based bottom-up methods can be listed: direct aqueous synthesis, one-step synthesis, micellar synthetic approaches, solvothermal approaches, high-temperature synthesis in organic solvents (organometallic and non-organometallic synthesis, microwave synthesis), two-phase approach, phase transfer method [21], [22]. Each approach has drawbacks and advantages. The solvothermal approach, which produces specified temperatures and pressures in an aqueous medium known as hydrothermal, makes it possible to quickly and efficiently create conditions that are ideal for the formation of nanocrystals [23]. High-quality nanocrystals were produced by organometallic synthesis in organic solvents, however because of their hydrophobicity, they cannot be used directly in biomedical applications. In order to produce water-soluble QDs, various successful investigations have been conducted in aqueous media [24]. Water-soluble QDs are produced using a more simple, affordable, environmentally friendly, and scale-able approach called the aqueous synthesis. However, because of the nature of the water, the aqueous approach results in flaws and contaminants on the QDs' surface; as a result, QDs with a wide size distribution and low fluorescence efficiencies can be produced. As a result, it has been preferred to synthesize highly fluorescent water-soluble QDs using metallic/organometallic precursors, capping, and surface modification. Different precursors are dissolved in distinct phases using the two-phase technique metallic/organometallic precursors and capping followed by surface modification [25]. The heat-up method, which offers a reaction that occurs in a single pot without requiring an injection phase, was found to be a high yielding methodology for the manufacturing of QDs [26]. A different strategy, known as the cluster-assisted method, focuses on using uniform seed/cluster particles as the nanocrystals' nuclei to regulate their size. This technique has shown a

flexible approach to concentration management and the capacity to produce a significant quantity of QDs in just one reaction system at a lower temperature [21]. The temperature adjustable synthesis of QDs is provided by the microwave-assisted technique. With good reproducibility, minuscule size errors, and a quick reaction time (within 3 min), our approach clearly outperformed the conventional synthesis method [27]. In this thesis, microwave assisted synthesis methods will be highlighted.

1.2.7. Characterization techniques of quantum dots

Characterization of QDs is essential for understanding their optoelectrical and physicochemical features, such as composition, shape, crystalline structure, bandgap energy, and surface ligand nature [28]. Optical characterization and structural characterization are the two main kinds of characterization methods for these nanoparticles The UV-Vis and fluorescence spectroscopy techniques are frequently used to characterize QDs optically, and they can give information on their concentration, size, and photoluminescence quantum yield [29], [30]. The shape, crystal structure, chemical content, and size of the semiconductor nanocrystals can all be understood through structural analysis of QDs using X-ray diffraction, X-ray photoelectron spectroscopy, transmission electron microscopy, and scanning electron microscopy [31]. Raman spectroscopy is also used by some research teams as it is a quick and accurate way to track changes in atom vibration [32].

1.2.8 Aflatoxin b1

Aflatoxins (AFs) are hazardous molecules that were first identified in British hatcheries in 1960 as a result of their toxicity to turkeys, chicks, and ducklings [33]. Aflatoxin B1 (AFB1) is thought to be the most harmful of the more than 20 known aflatoxins that infect a number of agricultural products. One of the mycotoxins made by *Aspergillus flavus* and *Aspergillus parasiticus*, aflatoxin B1 (AFB1) poses a serious risk to human health and contaminates food. AFB1's straightforward sensitivity makes its detection crucial and necessary for ensuring the quality and safety of food [34]. AFB1 is a biomolecule which possess fluorescence properties, besides functional groups in its chemical structure [35].

1.2.9 Fluorescence energy transfer

Fluorescence or Förster Resonance energy transfer (FRET) is the mechanism by which energy is transmitted from an excited donor particle to an acceptor particle. The FRET mechanism depends on decreasing the donor's emission and excited-state lifespan and increasing the intensity of the acceptor's emission. This occurs whenever the critical radius, also known as the Förster radius, is exceeded by the distance between the donor and acceptor. FRET is used to monitor changes in distances instead of absolute distances because it is sensitive to the distance between the donor and the acceptor. QDs have established themselves as FRET donors and acceptors, allowing for the assessment of biological systems. The peculiar characteristics of QDs, such as their large surfaces for the attraction of biomolecules, high brightness and photostability, strong and broad absorption, and color-tunability via QD size, shape, and material, have been the driving force behind this development [36]. Since organic dyes for FRET have early photobleaching issues and considerable emission overlap between the donor and acceptor, QDs are a great replacement. Applications for QD-based FRET technology include monitoring protein conformational changes, protein interactions, and assaying enzyme activity, quick and sensitive DNA detection, and DNA array analysis [37]. Whilst FRET is often computed by calculating the donor photoluminescence in the donor's absence (Figure 1.6 (A)) and in the donor's presence (Figure 1.6 (B)), it is also crucial to measure the acceptor's PL in the donor's presence when the donor is excited (Figure 1.6 (C)). It is strongly suggested that donor quenching was brought about by FRET to the acceptor and not by any other quenching processes by the sensitization of the acceptor to donor excitation [38].

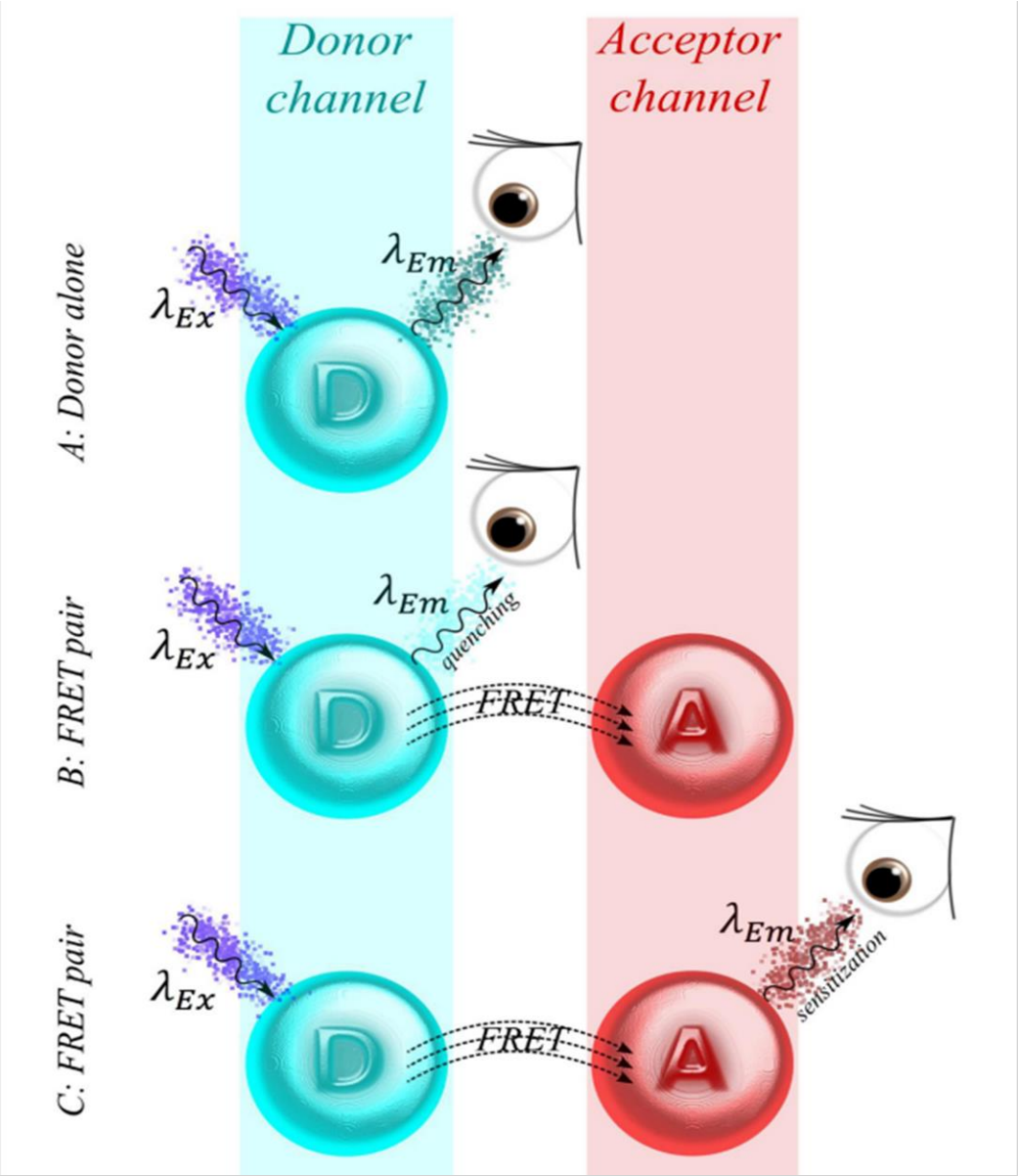

Figure 1.6 (A) Investigation of the donor's direct emission when there is no acceptor present. (B) Investigation of the donor quenching due to FRET in the existence of the acceptor. (C) Investigation the FRET-induced acceptor sensitization while the donor is present [38].

1.2.10 The purpose of the study

The purpose of the project is to synthesize yellow emissive carbon quantum dots using microwave assisted synthesis and interact them with AFB1, and demonstrate the extraordinary potential of carbon dots as an AFB1 chemosensor and the potential of AFB1 as an emission-intensity booster for yellow emissive carbon dots in biomedical applications such *in vivo* cell imaging.

2. METHOD

This section of the thesis provided in-depth explanations of the production and characterization of carbon quantum dots (PhDOTs and U:PhDOTs), as well as their interaction with the Aflatoxin B1 molecule.

2.1 Materials

High-purity 1-2 phenylenediamine (o-phenylenediamine) (Product code: Art. 809721) as a carbon precursor, urea (Cas-No: 57-13-6) as a passivating agent, and sodium hydroxide (Cas-No: 1310-73-2) were procured from Merck, while Aspergillus flavus aflatoxin B1 (Cas-No:1162-65-8) was obtained from Sigma-Aldrich Co.

2.2 Production of PhDOTs and U:PhDOTs

Carbon dots were synthesized from o-phenylenediamine (1-2 phenylenediamine) using a conventional microwave-assisted technique, illustrated in the Figure 2.1. First of all, PhDOTs were prepared by dissolving 400 milligrams of o-phenylenediamine as a carbon precursor with 20 milliliters of ultrapure water in a 50 milliliter round bottom flask, and the obtained homogenous solution was cooked at 800 MW on a revolving plate in a microwave oven (SAMSUNG/Model No: MS23F300EEK) designed for home use for 20 minutes. For second type of carbon dots (U:PhDOTs) were synthesized by dissolving 400 mg of o-phenylenediamine (a carbon precursor) and 100 mg of urea (a passivating agent) in 20 mL of ultrapure water and then the same proceeding was followed described above. As a result of the synthesis process, a yellowish solid material obtained and then, the solid product was dissolved in 10 mL of ultrapure water, and then large clusters were filtered out using a specific filter paper with a pore size of 10 μm before the solution was passed through a syringe filter with a pore size of 0.22 μm. Last but not least, the solvent was removed from the purified carbon quantum dot solution, and the resulting solid was stored at room temperature and out of direct light until further examination was performed.

2.3 Interaction of the Carbon QDs with Aflatoxin B1 Molecule

Carbon dot solutions were made with varying concentrations of AFB1 (10 g/mL, 8 g/mL, 4 g/mL, 2 g/mL, 1 g/mL, 0.5 g/mL, 0.25 g/mL, 0.125 g/mL, 0.62 ng/mL), then the AFB1 solutions were added to the carbon dot solutions (with the carbon dot concentration being constant). Then, carbon dots and AFB1 mixture waited 20 minutes for the complete interaction.

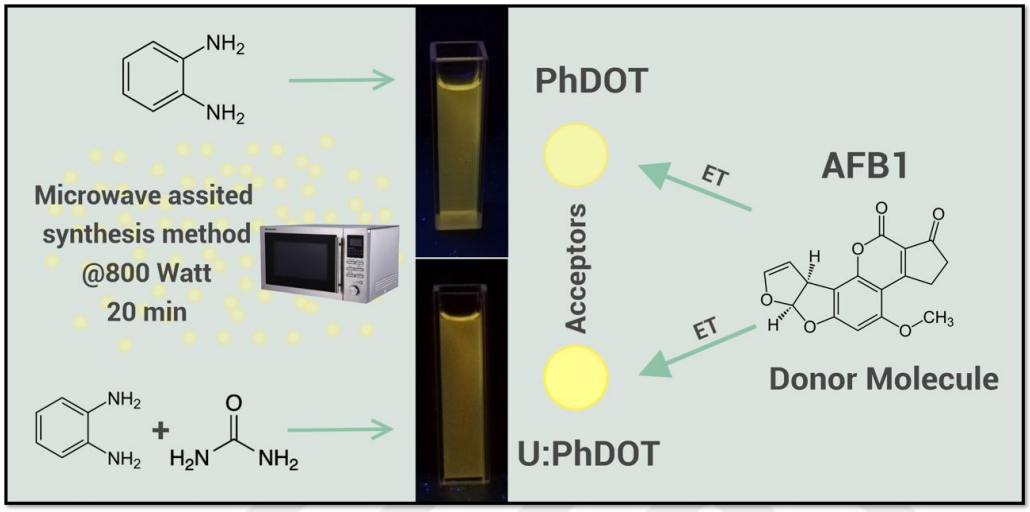

Figure 2.1: Schema of carbon dots synthesis and their UV (366 nm) irradiation-induced photoluminescence.

2.4 Optical and Structural Characterization of Carbon Dots, AFB1 and Carbon Dot - AFB1 Complexes

Fluorescence spectroscopy, UV-Vis spectroscopy, and time-resolved fluorescence spectroscopy were used to characterize the optical properties of AFB1, CDs, and CD - AFB1 complexes with in aqueous solutions and at room temperature. On the other hand, the structures of CDs were characterized with Fouirer transform infrared spectroscopy (FTIR) and Raman spectroscopy (RS). Scinco Neosys-2000 double-beam ultraviolet-visible (UV-Vis) spectrophotometer was used to record the absorption spectra of AFB, PhDOTs and U:PhDOTs carbon dots. To capture the emission spectra of carbon dots and AFB1, a Varian Cary Eclipse fluorescence spectrofluorometer was used with excitation wavelengths (λ_{exc}) ranging from 350 to 530 nm. After purification, each sample was dissolved in 10 mL of ultrapure water and diluted until the optical density was below 0.1; this was done to eliminate any mistakes introduced by self-absorption. For optical density and quantum yield calculation UV-vis spectrophotometer was utilized. Under 366 nm UV-light irradiation, the emission colors of carbon dots were analyzed. Using coumarine 102 in ethanol as a reference,

the quantum yields of PhDOTs and U:PhDOTs at 400 nm carbon dots were determined (QY:0.93) [39]. Photoluminescence excitation (PLE) spectra of both PhDOTs and U:PhDOTs were recorded at an emission wavelength (λ_{ems}) of 570 nm. Emission spectrum of each sample was recorded by using 350 nm, 400 nm, 450 nm and 530 nm excitation wavelengths. The photostability of carbon dots was measured by monitoring the change in intensity of emission at 570 nm upon continuous illumination with 350 nm for 1 hour. The emission properties of AFB1 and those of AFB1-carbon dots mixtures was compared. To do this, the emission spectra of each AFB1-carbon dots mixture was compared with the emission spectra of AFB1 at 400 nm which is excitation wavelength of carbon dots. In order to observe interaction between AFB1 and carbon QDs, emission and excitation spectra of AFB1-PhDOTs and AFB1-U:PhDOTs carbon dots mixtures were recorded. PicoQuant MicroTime hundred times resolved confocal fluorescence microscope was used to analyze the fluorescence kinetics of both PhDOTs and U:PhDOTs. The excitation beam was generated by a picosecond diode laser with 8 mW of power (excitation wavelength of 375 nm, pulse repetition rate of 60 MHz, and 40X objective). Bandpass filters with a bandwidth of 15 nm were employed to obtain spectrally resolved data. Furthermore, Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy (RS) were utilized to investigate the molecular composition of PhDOTs and U:PhDOTs CDs. The purified carbon dots were analyzed (in the range of 800-4000 cm^{-1}) by Perkin Elmer attenuated total reflectance FTIR (ATR-FTIR) spectrometer and Raman spectrometer to learn more about the bonding characteristics of PhDOTs and U:PhDOTs quantum dots.

3. RESULTS AND DISCUSSION

3.1 Production of PhDOTs and U:PhDOTs via Microwave Synthesis Method

Carbon dots are suitable agents for biomedical applications thanks to their low-toxicity [40]. It has been found that the fluorescence of blue-green spectrum carbon quantum dots have higher brightness, and their brightness is not powerful when they have yellow-red color. Even though it seems one of the carbon dot features, it raises a problem that needs to be overcome. Eukaryotic cells have fluorescence characteristics at the 350-550 nm, to put it another way blue-green color spectrum. This fact complicated the usage carbon dots in range of blue-green color for biological studies. As a result, research into and production of highly effective emissive carbon dots in the colors yellow and/or red is now cutting-edge challenge to achieve [41]. The emission color of carbon QDs can be adjusted by adjusting either their nanoparticle size or their nanoparticle surface features and composition. The conditions of bottom-up production technique make it difficult to regulate the size of carbon dots. Conversely, surface properties and composition of carbon dot nanoparticles are easily modifiable via bottom-up synthesis [42]. The most popular method for creating carbon dots with intense fluorescence, or high quantum yield (QY), among all forms of bottom-up synthesis approaches is microwave synthesis. While the microwave assisted synthesis of blue-green emissive carbon dots with high quantum yield (over 25%) is well-studied, the synthesis of yellow- or red-emitting emissive carbon dots is much less researched [43]. To create yellow-red emissive carbon dots, isomers of phenylenediamine are the favorite carbon precursors. However, for microwave-assisted synthesis of yellow emissive carbon dots, the greatest quantum yield was measured at 38.5% [44]**.** In this thesis, yellow emissive carbon dots were produced utilizing a standard technique previously published by Song et al.; 1-2 phenylenediamine by helping of urea and without urea (PhDOTs (without urea) and U:PhDOTs (with urea)) was heated at 800 W in a domestic microwave oven for 20 minutes, and carbon dots were formed [44]. By altering the synthesis protocol by adding urea to a solution of 1-2 phenylenediamine prior to microwave treatment, yellowish-emitting carbon dots may be produced. The structural and optical features

of carbon dots were studied extensively to enlighten the causes of the increase of the brightness of carbon dot emission.

3.2 Structural Characterization of PhDOTs and U:PhDOTs

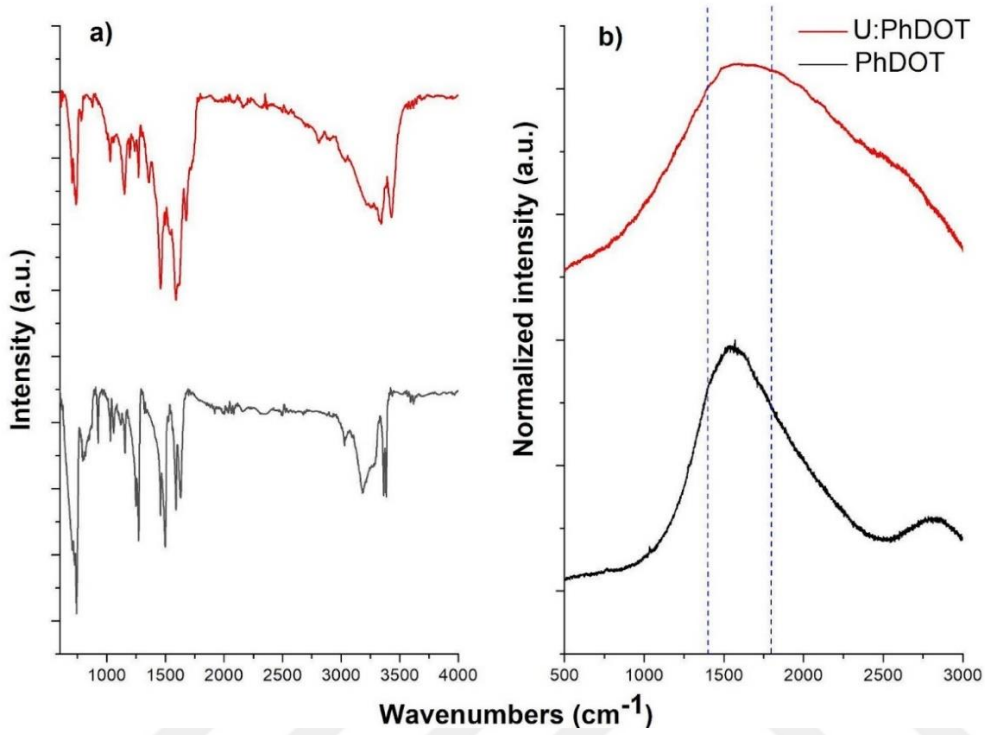

Figure 3.1 : (a) FTIR and (b) Raman spectrum of carbon quantum dots.

For structural characterization, Fourier transform infrared (FTIR) and Raman spectroscopies were used. The bonding characterization of core structure and the properties of functional groups on the surface of carbon QDs. The Figure 3.1 displays characteristic differentiation of FTIR and Raman spectrum of U:PhDOT and PhDOT carbon dots. In Figure 3.1 (a), one notable dissimilarity is that primary amines on the surface of the carbon dot are reflected in the FTIR spectra of U:PhDOT, whereas PhDOT does not show this peak at 3450 cm^{-1}. Nevertheless, the FTIR spectra of both U:PhDOTs and PhDOTs showed typical peaks corresponding to secondary amines (3300-3400 cm^{-1} band), carboxylic groups (1600-1700 cm^{-1} band), hydroxyl groups (3200-3300 cm^{-1} band), and C-N bonds (1000-1250 cm^{-1}). While both carbon dots featured nitrogen in their core structures (as evidenced by peaks corresponding to C-N bonds and secondary amine groups), only the U:PhDOTs has amine groups on their surfaces (as evidenced by peaks related to primary amine groups). It is important to note that the presence of carboxylic and hydroxyl groups on the surface of both types

of carbon dots was shown by the presence of FTIR peaks corresponding to these groups.

Figure 3.1 (b) presents Raman spectra of Ph:DOT and U:PhDOT with striking dissimilarities. Each carbon dot's Raman spectrum exhibited a D band at roughly 1400 cm^{-1} and a G band at around 1800 cm^{-1}, indicating that the carbon dots were graphitic in origin and had a considerable defect density on their surfaces. However, when comparing the raman spectra of U:PhDOT and PhDOT, the G:D ratio in the former was noticeably larger. Graphitic materials have a raman spectrum that reveals surface imperfections at the D band and an increase in the G band because of the presence of sp^2 hybridized carbons [44]. The oxygen on the surface of carbon dots, which manifests as hydroxyl and carboxyl groups, is primarily responsible for the emergence of defect energy states in these nanostructures. Based on these data, it was determined that the surface defect states of PhDOTs were much greater, as indicated by the existence of an intense D band in their Raman spectra, likely due to the predominance of carboxylic and hydroxyl groups on their surfaces, while the surface defect states of U:PhDOTs, which also contained amine groups, were lower, as indicated by the presence of an intense G band.

3.3 Optical Characterization of PhDOTs and U:PhDOTs

The optical characteristics of Ph:DOTs and U:PhDOTs were identical in their steady-state forms; for example, both types of carbon dots exhibited a single emission peak at 570 m with an FWHM of 85 nm. There was also not much variation in the absorption properties of the quantum dots; for example, the absorption spectra of the carbon dots all showed a gaussian peak at 440 nm, which corresponded to the band-edge transition; this peak is unusual for carbon-based quantum dots and may have arisen because of the creation of a rigid crystalline crystal structure [45]; and the absorption peaks at shorter wavelengths (below 300 nm) were typical for carbon dots.

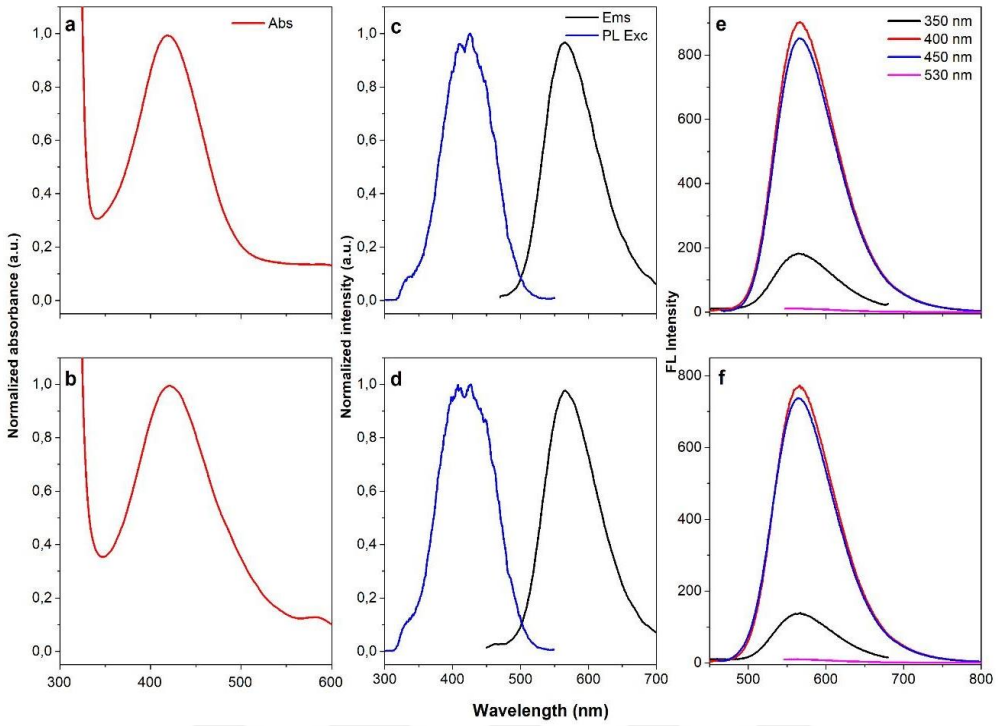

Figure 3.2 : UV-vis spectrum of (a) PhDOT and (b) U:PhDOT, PL spectra with λ_{exc} = 400 nm and PL spectrum with with λ_{ems} = 570 nm for (c) PhDOT, (d) U:PhDOT, PL spectra of (e) PhDOT, (f) U:PhDOT with different excitation wavelengths (λ_{exc} = 350, 400 and, 530 nm).

Photoluminescence excitation (PLE) spectra of both carbon dots were virtually identical; both PLE spectra featured a single, gaussian peak at 440 nm, an exact match for the band-edge transition peak in the absorption spectra of carbon dots (Figure 3.2 (a), (b)). Similarities between the photoluminescence emission (PLE) spectrum and the absorption spectrum demonstrated that the luminescence capabilities of Ph:DOTs and U:PhDOTs were mostly controlled by the inner structure of the carbon dot and enhanced because of band-edge electron transitions (Figure 3.2 (c), (d)). Emission qualities as a function of excitation were studied for both carbon dots to back up this claim. As a result of their unique surface properties, carbon dots of many varieties exhibit an excitation dependent emission property. Carbon dots' surface characteristics can be manipulated to regulate this characteristic [46]. The carbon dots had a single emission peak at 570 nm upon excitation at multiple wavelengths such as 350 nm, 400 nm, and 450 nm, but the PhDOTs and U:PhDOTs exhibited no excitation dependent emission characteristics (Figure 3.2 (e), (f)). Emission was strongest when excited at 400 nm, faded significantly at 450 nm, and was nearly eliminated when excited at 530 nm. While both carbon dots had nearly identical steady-state optical characteristics,

there was a large discrepancy in their quantum yields (QY). Upon excitation at 400 nm, the QY of U:PhDOT was determined to be 51%, while the QY of PhDOT was only 40%.

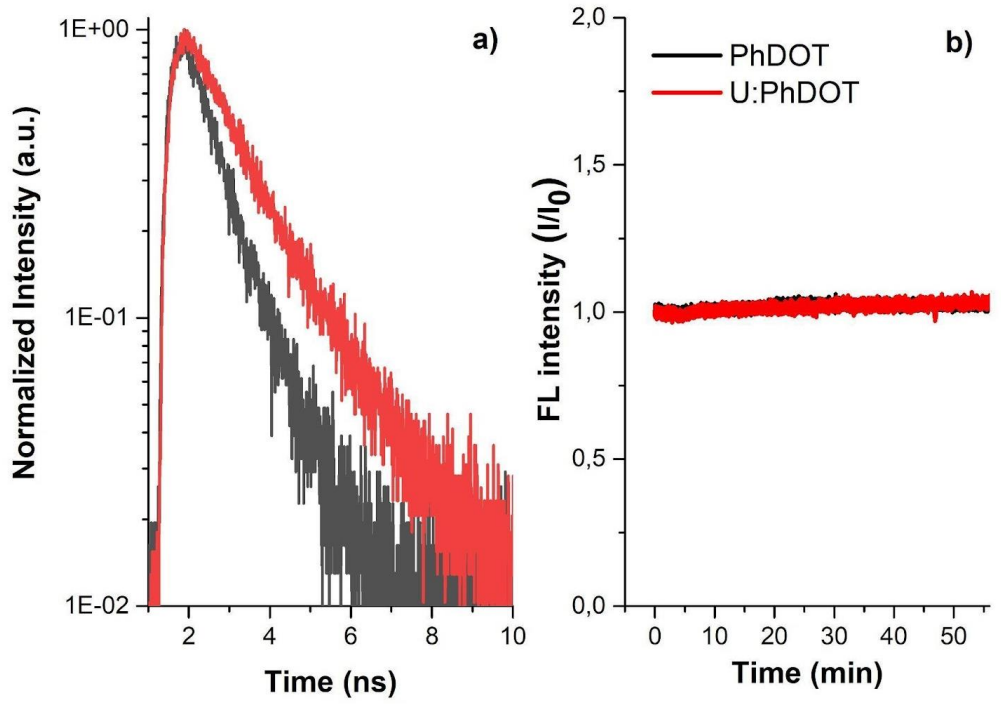

Figure 3.3 : (a) Fluorescence kinetics of CDs at 550 nm when excited at 375 nm. (b) photostability of CDs for 1 hour under continuous irradiation with exc=350 nm at room temperature.

Photostability and fluorescence kinetics of Ph:DOTs and U:Ph:DOTs were measured to help explain the significant difference between the two materials' QY (Figure 3.3 (a), (b)). Under constant illumination at 350 nm, both U:PhDOTs and PhDOTs remained photostable for at least an hour. Each carbon dot was stimulated at 375 nm and the number of photons emitted between 550 ± 15 nm later was counted to determine the fluorescence kinetics (Figure 3.3 (b)). The quantity of radiative recombination of the excitons detected as fluorescence can be inferred from a material's fluorescence lifetime, which is measured by observing the fluorescence kinetics of the material. There is more radiative decay in fluorescence with a longer lifetime, and less with a shorter one. The average fluorescence lifespan of PhDOTs was 1.1 ns, and their fluorescence degradation followed a single exponential distribution. However, the fluorescence lifespan of U:PhDOTs was roughly 1.8 ns, which was significantly longer than that of PhDOTs (Figure 3.3 (a)). The FTIR and

Raman spectra of U:PhDOTs corroborated these findings, demonstrating that the addition of urea during carbon dot synthesis led to surface passivation, which in turn reduced the number of defect states in the surrounding region of the carbon dots and increased the number of radiative combinations possible. That caused a massive boost in the U:PhDOTs' quantum yield.

Table 3.1 : Comparison of photophysical characteristics and lifetimes of AFB1, PhDOT, and U:PhDOT in their steady-state conditions.

	AFB1	PhDOT	U: PhDOT
QY	11%	40%	51%
τD	6 ns	1.1 ns	1.8 ns
Ems	440 nm	570 nm	570 nm
Abs	360 nm	420 nm	420 nm
FWHM	66 nm	85 nm	85 nm

As it can be seen from Table 1, because of its unique optical properties, aflatoxin B1 (AFB1) is an intriguing biomolecule. This thesis investigated the possibility of employing AFB1 as an energy donor to improve the quantum yield of carbon dots by examining its interaction with PhDOTs - U:PhDOTs. yield of carbon dots.

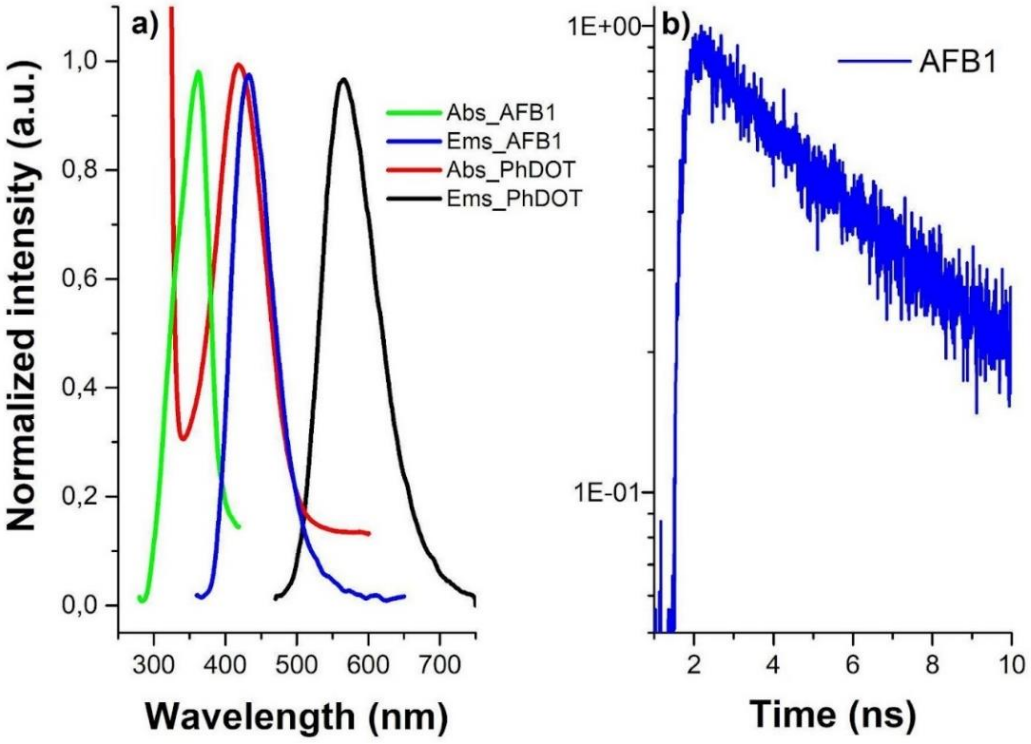

Figure 3.4 : (a) Emission and absorbance spectrum of AFB1 and PhDOT. (b) Fluorescence kinetics of AFB1 at 430 nm upon excitation at 375 nm.

First, the time-resolved and steady-state optical characteristics of AFB1 were analyzed in depth to get a full picture of the carbon dot-AFB1 interaction (Figure 3.4 (a), (b)). As AFB1 was stimulated at 350 nm, its emission spectrum showed a single emission peak at 440 nm, while its absorption spectra featured a single gaussian peak at 360 nm (Figure 3.4 (a)). According to the findings, the emission peak of AFB1 precisely matched with the band-edge transition peak in the absorption spectra of carbon dots and the PLE peak of carbon dots. The optical characteristics of AFB1 made it a promising candidate as a FRET donor for PhDOTs and U:PhDOTs, as evidenced by the exact overlap between its emission spectra and the absorption spectrum of carbon dots. Furthermore, photons emitted from the decay of AFB1 after being stimulated at 375 nm were measured in the 430 ± 15 nm emission range (Figure 3.4 (b)). As determined by studying the kinetics of AFB1's fluorescence, the average fluorescence lifespan of AFB1 is 6 ns.

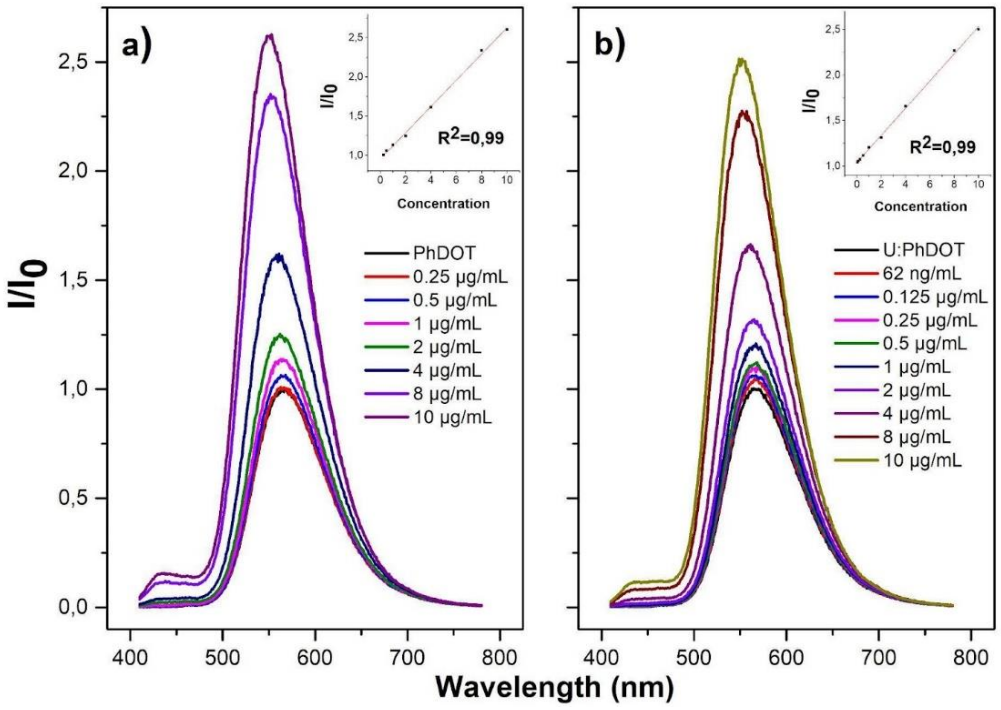

Figure 3.5 : PL spectrum of (a) PhDOT (20 mg/mL) - AFB1 ((0.25-10) µg/mL in DI water) interaction, (1:3-v/v). (b) U:PhDOT (20 mg/mL) - AFB1 (62 ng/mL-10 µg/mL in DI water) interaction, (1:3-v/v). All samples were excited at 400 nm. Insets of figures presents linear range where I is FL intensity of each component, I_0 is FL intesity of (a) PhDOT and (b) U:PhDOT.

The emission spectra of several carbon dots changed drastically as AFB1 was added to the solutions (Figure 3.5 (a), (b)). Each carbon dot type's emission intensity rose noticeably when AFB1 was present in the carbon dot solution at varying

concentrations. With a minimum concentration of 0.25 ug/mL AFB1, PhDOTs showed increased emission intensity. When AFB1 was present at a concentration greater than 0.25 g/mL, the emission intensity of PhDOT increased linearly (R^2=0.99), and it increased by a factor of 2.65 when the AFB1 concentration reached 10 g/mL. Moreover, at 10 g/mL AFB1, the emission peak of PhDOT migrated somewhat toward lower wavelengths, and the emission peak of PhDOTs was found at 548 nm. The AFB1 - U:PhDOT interaction was similar, but with a few key distinctions. The AFB1 - PhDOT interaction was shown to result in a linear increase (R^2=0.99) in emission intensity for U:PhDOTs of 2.45 fold at a concentration of 10 g/mL AFB1. While a minimum of 62 ng/mL of AFB1 was required to see the rise in emission intensity, higher concentrations were seen with higher concentrations of the molecule.

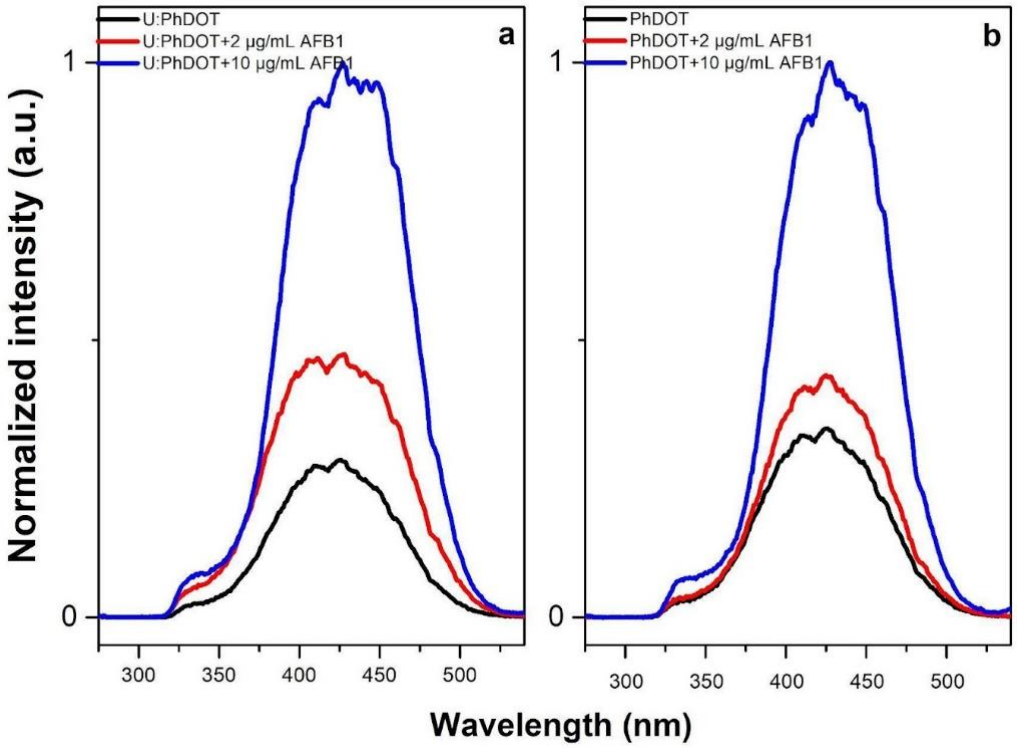

Figure 3.6 : Excitation spectra for (a) U:PhDOT at 570 nm, U:PhDOT + 2 µg/mL AFB1 in water at 566 nm and U:PhDOT + 10 µg/mL AFB1 in water at 555 nm. (b) PhDOT at 570 nm (maximum emission wavelength), PhDOT + 2 µg/mL AFB1 in water at 564 nm, PhDOT + 10 µg/mL AFB1 in water at 548 nm.

To figure out why yellow-emitting carbon dots fluoresced more after interacting with AFB1, steady-state PLE spectra and fluorescence kinetics were measured at emission wavelengths of 430 ± 15 nm (AFB1 emission) and 550 ± 15 nm (carbon dots emission) for carbon dot-AFB1 complexes (Figure 3.6 (a), (b)). As the amount of

AFB1 in the carbon dot solution went up, the peak in the PLE spectrum of carbon dots got stronger. These results showed that when AFB1 was present, the number of radiative transitions gradually went up. But steady state fluorescence spectroscopy couldn't provide a more detailed explanation of why the fluorescence of carbon dots is getting brighter. It's possible that the AFB1 molecules are further passivating the surface of the carbon dots, leading to an increase in the quantum yield (as was shown in the U:PhDOT example), or that they are acting as an energy donor, leading to an increase in the quantum yield of the carbon dots. So, fluorescence kinetics of PhDOT-AFB1 and U:PhDOT-AFB1 complexes were measured to find out why the quantum yield of carbon dots went up.

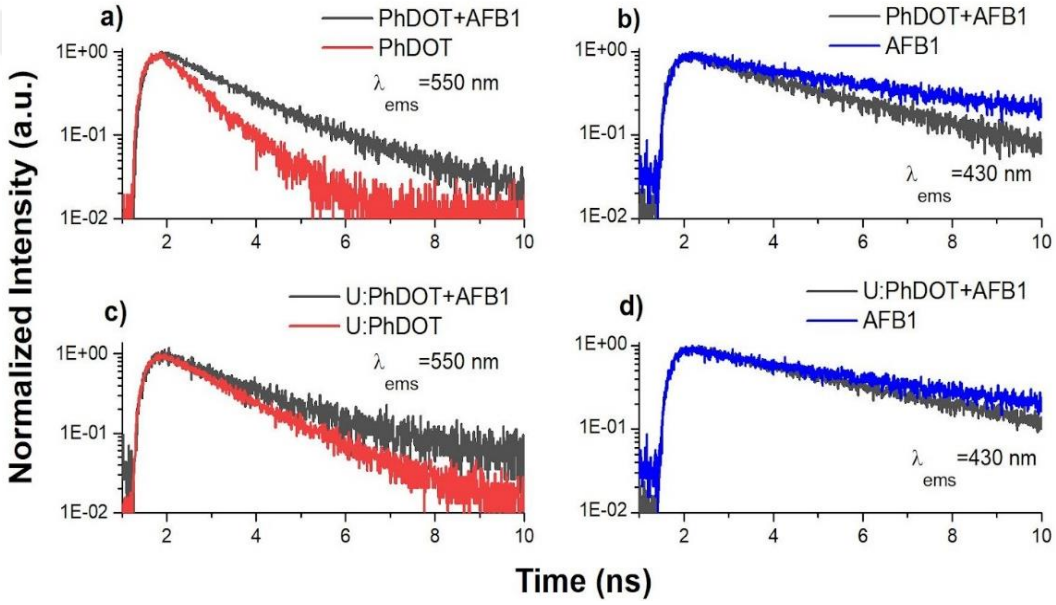

Figure 3.7 : Fluorescence kinetics of (a) PhDOT+AFB1 mixture and PhDOT at 550 nm, (b) PhDOT+AFB1 mixture and AFB1 at 430 nm (c) U:PhDOT+AFB1 mixture and U:PhDOT at 550 nm (d) U:PhDOT+AFB1 mixture and AFB1 at 430 nm. All samples were excited at 375 nm.

To fully understand how energy is transferred, the fluorescence decays were measured at two different lengths of light (Figure 3.7). To figure out how the fluorescence kinetics of carbon dots change, first the fluorescence decays at 550 ± 15 nm, which is where carbon dots emit light, were measured. The average fluorescence lifetime of PhDOTs was 1.1 ns before adding AFB1 and 2 ns after adding AFB1. For U:PhDOTs, the average fluorescence lifetime was 1.8 ns before adding AFB1 and 2.3 ns after adding AFB1. This showed that the number of radiative transitions increased after adding AFB1 and confirmed the results from PLE spectra of carbon dot + AFB1

complexes. However, in carbon dots + AFB1 complexes, the fluorescence lifetime of AFB1 was drastically reduced as determined by measuring fluorescence decay at an emission wavelength of 430 ± 15 nm (The average fluorescence lifetimes; for PhDOT + AFB1 mixture: 3.5 ns, U:PhDOT + AFB1 mixture: 4 ns, only AFB1: 6 ns). The reduction in radiative transitions at 430 ± 15 nm, which were transferred to the carbon dots and generated a considerable rise in radiative transitions at 550 ± 15 nm, was the primary factor in reducing the lifetime of AFB1 in carbon dots + AFB1 complexes. The energy transfer efficiency (E) was evaluated d by using equation 3.1:

$$E = 1 - \frac{\tau D}{\tau DA} \tag{3.1}$$

where τ_{DA} was the average fluorescence lifetime of AFB1 in carbon dot + AFB1 mixtures and τ_{DA} was fluorescence of AFB1 alone. When comparing the two pairs, the E value for PhDOT+AFB1 was 0.42 whereas the value for U:PhDOT + AFB1 was 0.33. These findings confirmed the existence of energy transfer between carbon dots and AFB1, regardless of carbon dot type, but with a little reduction in efficiency due to the presence of amine groups on carbon dots.

4. CONCLUSION

This thesis study demonstrates that quantum yield enhancement of conventional yellow emissive carbon dots by passivating the surface of the carbon dots with urea via microwave-assisted production method. While surface passivation had no discernible effect on the steady state emission properties of carbon dots, it did dramatically improve their quantum yield and average fluorescence lifespan. The interaction between AFB1 and the yellow emissive carbon dots resulted in a considerable boost in the emission intensity of the carbon dots. By measuring the fluorescence lifetime, it was found that AFB1 served as an energy donor for carbon dots, leading to a notable boost in fluorescence lifetime and quantum yield. These findings not only demonstrated the promising future of yellow emissive carbon dots as a chemosensor for AFB1, but also suggested that AFB1 might be employed as a possible emission-intensity-booster for yellow emissive carbon dots in biomedical applications such *in-vivo* cell imaging.

REFERENCES

[1] **Gnach, A., Lipinski, T., Bednarkiewicz A., Rybka J. and Capobianco, J. A.** (2015). Upconverting nanoparticles: Assessing the toxicity, *Chem. Soc. Rev.*, 44, 6, 1561–1584.

[2] **Gidwani B. et al.** (2021). Quantum dots: Prospectives, toxicity, advances and applications, *J. Drug Deliv. Sci. Technol.*, 61, 102308.

[3] **Reshma, V. G. and Mohanan, P. V.** (2019). Quantum dots: Applications and safety consequences, *J. Lumin.*, 205, 287–298.

[4] **Ekimov, A. I, Efros, A. L. and Onushchenko, A. A.** (1985). Quantum size effect in semiconductor microcrystals, *Solid State Commun.*, 56, 11, 921–924,

[5] **Rossetti, R. and Brus, L.** (1982). Electron-hole recombination emission as a probe of surface chemistry in aqueous CdS colloids, *J. Phys. Chem.*, 86, 23, 4470–4472.

[6] **Weller H.** (1993). Colloidal Semiconductor Q- Particles: Chemistry in the Transition Region Between Solid State and Molecules, *Angew. Chemie Int. Ed. English*, 32, 1, 41–53.

[7] **Murray, C. B., Norris D. J., and Bawendi M. G.** (1993) Synthesis and Characterization of Nearly Monodisperse CdE (E = S, Se, Te) Semiconductor Nanocrystallites, *J. Am. Chem. Soc.*, 115, 19, 8706–8715.

[8] **Rani, U. A., Ng, L. Y., Ng, C. Y., and Mahmoudi J.** (2020). A review of carbon quantum dots and their applications in wastewater treatment, *Adv. Colloid Interface Sci.*, 278, 102124.

[9] **AbouElhamd, A. R., Al-Sallal, K. A. and Hassan, A.** (2019). Review of core/shell quantum dots technology integrated into building's glazing, *Energies*, 12, 6.

[10] **Wang, J., Han, S., Ke, D. and Wang, R.** (2012). Semiconductor quantum dots surface modification for potential cancer diagnostic and therapeutic applications, *J. Nanomater.*, 2012.

[11] **Shen, H. et al.** (2017). Efficient and long-lifetime full-color light-emitting diodes using high luminescence quantum yield thick-shell quantum dots, *Nanoscale*, 9, 36, 13583–13591.

[12] **García de Arquer, F. P., Talapin, D. V., Klimov, V. I., Arakawa, Y., Bayer, M. and Sargent, E. H.** (2021). Semiconductor quantum dots: Technological progress and future challenges, *Science*, 373, 6555.

[13] **Morozova, S., Alikina, M., Vinogradov, A. and Pagliaro, M.** (2020). Silicon Quantum Dots : Synthesis , Encapsulation , and Application in Light-Emitting Diodes, 8, 1–8.

[14] **Das, A. and Snee, P. T.** (2016). Synthetic Developments of Nontoxic Quantum Dots, *ChemPhysChem*, 17, 5, 598–617.

[15] **Budak, E., Erdoğan, D. and Ünlü, C.** (2021). Enhanced fluorescence of photosynthetic pigments through conjugation with carbon quantum dots, *Photosynth. Res.*, 147, 1, 1–10.

[16] **Namdari, P., Negahdari, B. and Eatemadi, A.** (2017). Synthesis, properties and biomedical applications of carbon-based quantum dots: An updated review," *Biomed. Pharmacother.*, 87, 88, 209–222.

[17] **Desmond, L. J., Phan, A. N. and Gentile, P.** (2021). Critical overview on the green synthesis of carbon quantum dots and their application for cancer therapy, *Environ. Sci. Nano*, 8, 4, 848–862.

[18] **Ergüder, Ö., Şahin Keskin, S., Nar, I., Trabzon, L. and Ünlü, C.** (2022). Aflatoxin B1 Acts as an Effective Energy Donor to Enhance Fluorescence of Yellow Emissive Carbon Dots, *ACS Omega*, 7, 33, 29297–2930.

[19] **Pillar-Little, T. J., Wanninayake, N., Nease, L., Heidary, D. K., Glazer, E. C. and. Kim, D. Y.** (2018). Superior photodynamic effect of carbon quantum dots through both type I and type II pathways: Detailed comparison study of top-down-synthesized and bottom-up-synthesized carbon quantum dots, *Carbon N. Y.*, 140, 616–623.

[20] **Valizadeh A. et al.** (2012). Quantum dots: Synthesis, bioapplications, and toxicity, *Nanoscale Res. Lett.*, 7, 19–21.

[21] **Lutfan Sinatra, J. P. O. M. B.** (2017). Methods of Synthesizing Monodisperse Colloidal Quantum Dots, *Mater. Matters*,12.

[22] **Kim, J. Y., Voznyy, O., Zhitomirsky, D. and Sargent, E. H.** (2013). 25th anniversary article: Colloidal quantum dot materials and devices: A quarter-century of advances, *Adv. Mater.*, 25, 36, 4986–5010.

[23] **Bajorowicz, B., Kobylański, M. P., Gołąbiewska, A, Nadolna, J., Zaleska-Medynska, A. and Malankowska, A.** (2018). Quantum dot-decorated semiconductor micro- and nanoparticles: A review of their synthesis, characterization and application in photocatalysis, *Adv. Colloid Interface Sci.*, 256, 352–372.

[24] **Hu, L. et al.** (2016). Metal-based quantum dots: Synthesis, surface modification, transport and fate in aquatic environments and toxicity to microorganisms, *RSC Adv.*, 6, 82, 78595–78610.

[25] **Hu, M. Z. and Zhu, T.** (2015). Semiconductor Nanocrystal Quantum Dot Synthesis Approaches Towards Large-Scale Industrial Production for Energy Applications, *Nanoscale Res. Lett.*, 10, 1, 1–15.

[26] **Kwon, S. G. and Hyeon, T.** (2011). Formation mechanisms of uniform nanocrystals via hot-injection and heat-up methods, *Small*, 7, 19, 2685–2702.

[27] **Zhou D. et al.** (2018). Microwave-Assisted Heating Method toward Multicolor Quantum Dot-Based Phosphors with Much Improved Luminescence, *ACS Appl. Mater. Interfaces*, 10, 32, 27160–27170.

[28] **Pan, Y., Li, Y. R., Zhao, Y. and Akins, D. L.** (2015). Synthesis and Characterization of Quantum Dots: A Case Study Using PbS, *J. Chem. Educ.*, 92, 11, 1860–1865.

[29] **L. Pan et al.** (2017). Highly efficient Z-scheme WO3-x quantum dots/TiO2 for photocatalytic hydrogen generation, *Cuihua Xuebao/Chinese J. Catal.*, 38, 2, 253–259.

[30] **Wang, Y., Ma, Q., Jia, H. and Wang, Z.** (2016). One-step solution synthesis and formation mechanism of flower-like ZnO and its structural and optical characterization, *Ceram. Int.*, 42, 9, 10751–10757.

[31] **Tajik, S. et al.** (2020). Carbon and graphene quantum dots: A review on syntheses, characterization, biological and sensing applications for neurotransmitter determination, *RSC Adv.*, 10, 26, 15406–15429.

[32] **Ortiz, S. N. C., Ospino, E. M. and Cabanzo, R.** (2016). Spectroscopy characterization and quantum yield determination of quantum dots, *J. Phys. Conf. Ser.*, 687, 1.

[33] **Richard. J. L.** (2008). Discovery of aflatoxins and significant historical features, *Toxin Rev.*, 27, 42067, 171–2010.

[34] **Luo, Y., Liu, X. and Li. J.** (2018). Updating techniques on controlling mycotoxins - A review, *Food Control*, 89, 123–132.

[35] **V V, A. K. Y., Renuka, R. M. andAchuth, J.** RSC Advances Development of a FRET-based fl uorescence aptasensor for the detection of a fl atoxin B1 in contaminated food grain samples, *RSC Adv.*, 8, 10465–10473.

[36] **Chou, K. F. and Dennis, A. M.** (2015). Förster resonance energy transfer between quantum dot donors and quantum dot acceptors, *Sensors (Switzerland)*, 15, 6, 13288–13325.

[37] **Rizvi, S. B., Ghaderi, S., Keshtgar, M. and Seifalian, A. M.** (2010). Semiconductor quantum dots as fluorescent probes for in vitro and in vivo bio-molecular and cellular imaging, *Nano Rev.*, 1, 1, 5161, 2010.

[38] **Cardoso Dos Santos, M., Algar, W. R., Medintz, I. L. and Hildebrandt, N.** (2020). Quantum dots for Förster Resonance Energy Transfer (FRET), *TrAC - Trends Anal. Chem.*, 125, 115819.

[39] **Ünlü C.** (2019). Controlling defect state emission in ultra-small sized tellurium doped CdSe nanocrystals via two-phase synthesis method, *Opt. Mater. (Amst).*, 89, 361–367.

[40] **Behi, M., Gholami, L., Naficy, S., Palomba, S. and Dehghani, F.** (2022). Carbon dots: a novel platform for biomedical applications, *Nanoscale Adv.*, 4, 353-376.

[41] **Zhu, S., Song, Y., Zhao, X., Shao, J., Zhang, J. and Yang, B.** (2015). The photoluminescence mechanism in carbon dots (graphene quantum dots, carbon nanodots, and polymer dots): current state and future perspective, *Nano Res.*, 8, 2, 355–381.

[42] **Shen J., Zhu, Y., Yang, X. and Li, C.** (2012). Graphene quantum dots: Emergent nanolights for bioimaging, sensors, catalysis and photovoltaic devices, *Chem. Commun.*, 48, 31, 3686–3699.

[43] **Budak E., Aykut S., Paşaoğlu, M. E. and Ünlü, C.** (2020). Microwave assisted synthesis of boron and nitrogen rich graphitic quantum dots to enhance fluorescence of photosynthetic pigments, *Mater. Today Commun.*, 24.

[44] **Song, L., Cui, Y., Zhang, C., Hu, Z. and Liu, X.** (2016). Microwave-assisted facile synthesis of yellow fluorescent carbon dots from o-phenylenediamine for cell imaging and sensitive detection of Fe3+ and H2O2," *RSC Adv.*, 6, 21, 17704–17712, 2016.

[45] **Coşkun Y. et al.** (2022). Development of Highly Luminescent Water-Insoluble Carbon Dots by Using Calix[4]pyrrole as the Carbon Precursor and Their Potential Application in Organic Solar Cells, *ACS Omega*, 7, 22, 18840–18851.

[46] **Gencer, O., Çeven, Ö. F. and Ünlü, C.** (2022). Triggering excitation independent fluorescence in zinc(II) incorporated carbon dots: Surface passivation of carbon dots with zinc(II) ions by microwave assisted synthesis methods, *Diam. Relat. Mater.*, 123, 108874.

CURRICULUM VITAE

Name Surname: Özge ERGÜDER

EDUCATION

B.Sc. Degree: 2019, Yıldız Technical University, Bioengineering Department

M.Sc. Degree: 2022, Istanbul Technical University, Nano Science and Nano Engineering Programme

PRESENTATIONS ON THE THESIS

Ergüder, Ö., Şahin, S., Ünlü, C., Trabzon, L. (2021). Development of Highly Luminescent CdTeS Quantum dots Through Two-Phase Synthesis Approach, 4th *International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem 2021),* Ankara, Turkey

PUBLICATIONS ON THE THESIS

Ergüder, Ö., Şahin Keskin, S., Nar, I., Trabzon, L. and Ünlü, C. (2022). Aflatoxin B1 Acts as an Effective Energy Donor to Enhance Fluorescence of Yellow Emissive Carbon Dots, *ACS Omega*, 7, 33, 29297–2930.