

CARBON DOTS WITH BIOMASS RED ALGA *KAPPAPHYCUS ALVAREZII* EXTRACT: PREPARATION, CHARACTERIZATION AND DRUG DELIVERY APPLICATION

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Engineering/Technology degree in
Sathyabama Institute of Science and Technology

by

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(DEEMED TO BE UNIVERSITY)**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of ANITHA V (39230003), SANJANA M(39230038 who carried out project entitled "CARBON DOTS WITH BIOMASS RED ALGA *KAPPAPHYCUS ALVAREZII* EXTRACT: PREPARATION, CHARACTERIZATION AND DRUG DELIVERY APPLICATION" under my / our supervision from September 2022 to April 2023.

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ABSTRACT

Over recent decades, great advances have been made in the combination of nanotechnology and medicine, which is paving the way towards the goal of biological applications. A large amount of biocompatible fluorescent nanomaterials such as quantum dots, metal nanoclusters and fluorescent polymers have been developed. Carbon dots are currently emerging as a class of promising fluorescent probe on account of their low photo bleaching, no optical blinking, tunable photoluminescence, versatile surface and excellent biocompatibility. *Kappaphycus alvarezii* is used to synthesize carbon dots because it contains high amount of natural carbon. Carbon dots are synthesized from carbon rich sources, and *Kappaphycus alvarezii* is one such source that is readily available and sustainable. These excellent properties of carbon dots have made bright prospects in the application of bio-imaging, drug delivery, bio-chemical detection, and sensors. Currently, intensive research has been focusing on the synthesis of carbon dots with high quantum efficiency and the construction of multifunctional systems based on carbon dots. We are reporting on a highly economical seaweed-based hydrothermal method for one-pot green synthesis of water dispersible fluorescent carbon dots by using seaweed *Kappaphycus alvarezii* extract as precursor. The synthesized carbon dots were characterized by UV-visible, fluorescence, Fourier transform infrared (FT-IR), dynamic light scattering (DLS) and transmission electron microscopic (TEM). The carbon dots are well dispersed in water with an average size of ~15 nm and show bright blue fluorescence under UV-light. These carbon dots conjugated with penicillin/streptomycin and acted as excellent fluorescent probes in cellular imaging of *Escherichia coli* and *Staphylococcus aureus*.

Keywords: Carbon Nanomaterials, Carbon Dots, Biological Applications, Drug delivery, Bioimaging, Biosensors.

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LIST OF ABBREVIATIONS

S.NO	ABBREVIATIONS	FULL FORM
01	CNT	Carbon nanotube
02	GR	Graphene
03	GO	Graphene Oxide
04	SWCNT	Single Walled Carbon NanoTubes
05	MWCNT	Multi Walled Carbon NanoTubes
06	DWCNT	Double Walled Carbon NanoTubes
07	CD	Carbon Dots
08	CA	Citric Acid
09	TADF	Thermally Activated Delayed Fluorescence
10	RTP	Room Temperature Phosphorescence
11	QY	Quantum Yield
12	GQD	Graphene Quantum Dots
13	CVD	Chemical Vapour Deposition
14	EGFP	Enhanced Green Fluorescent Protein
15	UV	Ultra Violet
16	XRD	X-Ray Diffraction
17	PL	Photo Luminescence
18	FTIR	Fourier Transform Infrared Spectroscopy
19	TEM	Transmission Electron Microscopy
20	DLS	Dynamic Light Scattering
21	LB	Luria Bertani
22	E.Coli	Escherichia coli
23	S.aureus	Staphylococcus aureus
24	nm	Nanometer

CHAPTER 1

INTRODUCTION

Nanoscience and technology is one of the most important technologies in the 21st century. Currently, nanotechnology has been widely applied in enormous fields, such as biological, biomedical, and chemical analysis. In recent years, fluorescent nanomaterials have garnered much interest as potential competitors to conventional fluorescent dye probe and developed quite rapidly due to the extensive demand of fluorescent probe in chemical sensors, biological monitoring, and other connected fields. Compared with the traditional fluorescent dye, fluorescent nanomaterials have quantum size effect and the distinctive effects of nanomaterials, which can overcome many shortcomings of the end, such as the low stability, weak fluorescence intensity, and fast photo-bleaching. As a result, fluorescent nanomaterials have been applied widely in physical, biological, and chemistry as well as other fields (Jun Zuo *et al.*, 2015). Among many kinds of nanoparticles, semiconductor quantum dots have become the pivot of attention due to their unique electronic and luminescent properties. Although, semiconductor quantum dots usually contain heavy metals, which are generally substantial toxic and limit their further applications in the field of biomedicine. (Clapp *et al.*, 2007). Thus, the development of new nontoxic heavy metal-containing nanomaterials is one of the current modes.

In the field of nanoscience and technology, carbon-based nanomaterials (CBNs) are becoming attractive nanomaterials (Cha *et al.*, 2013; Wang *et al.*, 2014, 2015; Tiwari *et al.*, 2015; Lin *et al.*, 2016; Mukhopadhyay *et al.*, 2016; Zhang *et al.*, 2017). Due to the existence of diverse carbon allotropes, from renowned allotropic phases such as amorphous carbon, graphite and diamonds to newly discovered carbon nanotubes (CNTs), graphene oxide (GO), graphene quantum dots (GQDs) and fullerene, carbon-based materials have recently become prized. Each member of the carbon family displays inimitable features and has been widely exploited in varied biological applications including biosensing, drug delivery, tissue engineering, imaging, diagnosis and cancer therapy (Hong *et al.*, 2015;

Bhattacharya *et al.*, 2016). During the relative brief time since the discovery of fullerenes in 1985, carbon nanotubes in 1991, and graphene in 2004, the unique properties of carbon-based nanomaterials have attracted great attentiveness, which has advanced the development of methods of large -scale industrial production. The continuously booming commercial use of engineered carbon-based nanomaterials includes technical, medical, environmental, and agricultural uses. Regardless of the application field, this is also associated with an increasing trend of intentional or unintended discharge of carbon nanomaterials into the environment, where the effect of living organisms is still challenging to predict (Maiti *et al.*, 2019).

In recent years, several researchers have devoted their time on the preparation of fluorescent carbon dots emitters without the use of organic chemicals in a single step, which is called as green chemistry concept (Li *et al.*, 2011; Fang *et al.*, 2011). To support this assumption, several green synthetic approaches have been developed for the preparation of carbon dots by using inexpensive renewable resources as the precursor. With the inspiration of these achievements, In the present study, green chemistry synthetic approach for the preparation of water-soluble fluorescent carbon dots in a single step technique using the marine red alga *Kappaphycus alvarezii* extract as a rich carbon source (Zhang *et al.*, 2011)

CHAPTER 2

2.LITERATURE SURVEY

2.1 Carbon Nanomaterials

In general, nanomaterials are defined as materials containing particles with one dimension between 1 and 100 nm in size. All nanomaterials composed of carbon atoms are termed carbon-based or carbon nanomaterials. Until recently, only two natural carbon allotropes were familiar: diamond and graphite. Meanwhile, various new allotropic forms have been described, including carbon nanomaterials. Carbon is one the most versatile element in the periodic table , owing to the large number of bonds of different type and strength that can form with it or with many other elements (Rauti *et al.*, 2019). Moreover, the capacity of carbon orbitals to hybridize in sp , sp^2 and sp^3 configurations paves the way to the existence of a number of allotropes. To date, the three naturally occurring allotropes of carbon are (diamond, amorphous carbon and graphite), (Pantarotto *et al.*, 2004) have been joined by additional ones deriving from synthetic processes (such as graphene, carbon nanotubes, fullerenes, carbon nano-horns, nano-diamonds[Fig. 2.1].

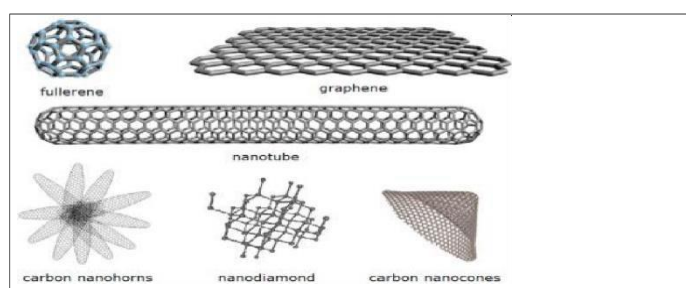


Fig. 2.1. Carbon allotropes derived from synthetic process.

The interest in carbon dots has widely increased exponentially in the last decades, first with the findings of Fullerenes (1985), then with that of Carbon nanotubes (CNTs; 1991) and finally with the synthesis of Graphene (GR). The properties of these CBNs make it widely used in many fields ranging from material science, energy production and storage, environmental sciences, biology and medicine.

2.2 Classification of Carbon Nanomaterials

Classification of carbon-based or carbon nanomaterials is most performed according to their geometrical structure. Carbon nanostructures include particles which are tube-shaped, horn-shaped, spherical, or ellipsoidal. Nanoparticles holding the shape of tubes are termed as carbon nanotubes; horn-shaped particles are nano horns and spheres or ellipsoids belong to the group of fullerenes.

2.2.1 FULLERENES

The examination of carbon nanostructures began with the discovery of the fullerenes. In general, fullerenes look like closed hollow cages made of sp^2 -hybridized carbon atoms arranged into 12 pentagons and a determined number of hexagons that depends on the total number of carbon atoms. Fullerenes are an allotropic adaptation of carbon, often termed as a molecular form of carbon, or carbon molecules. Fullerenes were discovered in 1985 by (Kroto *et al.*,1985) who were awarded later with the nobel prize for chemistry in 1996. A fullerene of $20 + 2n$ carbon atoms will have n hexagons. The number of pentagons is predetermined by the closed shapes of the fullerenes and will always be 12 in those with perfect structures. The fullerene family made up of several atomic C_n clusters ($n > 20$), composed of carbon atoms on a spherical surface. Carbon atoms are normally located on the surface of the sphere at the vertices of pentagons and hexagons. In fullerenes, carbon atoms are typically present in the sp^2 -hybrid form and connected by covalent bonds. C_{60} is a simple spherical molecule with an external diameter of 0.71 nm, and its chemical characters are very similar to those of an organic molecule. However, it can also be regarded as the smallest carbon nanostructure and a representative 0D carbon nano-allotrope. Fullerene C_{60} is the most analyzed and best-investigated fullerene. The spherical molecule is highly symmetric and consists of 60 carbon atoms, located at the vertices of twenty hexagons and twelve pentagons (Yadav *et al.*,2008).

2.2.2 CARBON NANOTUBES (CNTs)

Among other carbon-based nanomaterials, CNTs are one of the carbon allotropes with exceptional properties to explore for technical applications. Carbon nanotubes are characterized by cylindrical structures with a diameter of several nanometers, consisting of rolled graphene sheets (Fig.2.2). Carbon nanotubes may

differ in length, diameter, chirality (symmetry of the rolled graphite sheet) and the number of layers. According to their structure, CNTs may be classified into two main groups: single-walled nanotubes (SWCNTs) and multi-walled nanotubes (MWCNTs). Some researchers additionally isolate double-walled carbon nanotubes (DWCNTs) as a separate class of CNTs.

Generally, SWCNTs have a diameter of around 1–3 nm and a length of a few micrometers. Multi-walled CNTs have a diameter of 5–40 nm and a length of around 10 μm . However, currently synthesis of CNTs with a length of even 550 mm has been reported (Zhang, *et al.*, 2013). The structure of CNTs leads to extraordinary properties with a unique combination of rigidity, strength and elasticity compared with other fibrous materials. For instance, CNTs exhibit considerably higher aspect ratios (length to diameter ratios) than other materials, and larger aspect ratios for SWCNTs as compared with MWCNTs due to their smaller diameter. Additionally, CNTs show high thermal and electrical conductivity compared to other conductive materials. Electrical properties of SWCNTs are based on their chirality or hexagon orientation with respect to the tube axis. Accordingly, SWCNTs are divided into three sub-classes: (i) armchair (electrical conductivity > copper), (ii) zigzag (semi-conductive properties) and (iii) chiral (semi-conductive properties) (Fig. 2.2e). By contrast, MWCNTs consisting of multiple carbon layers, frequently with variable chirality, can exhibit outstanding mechanical properties instead of exceptional electrical characteristics.

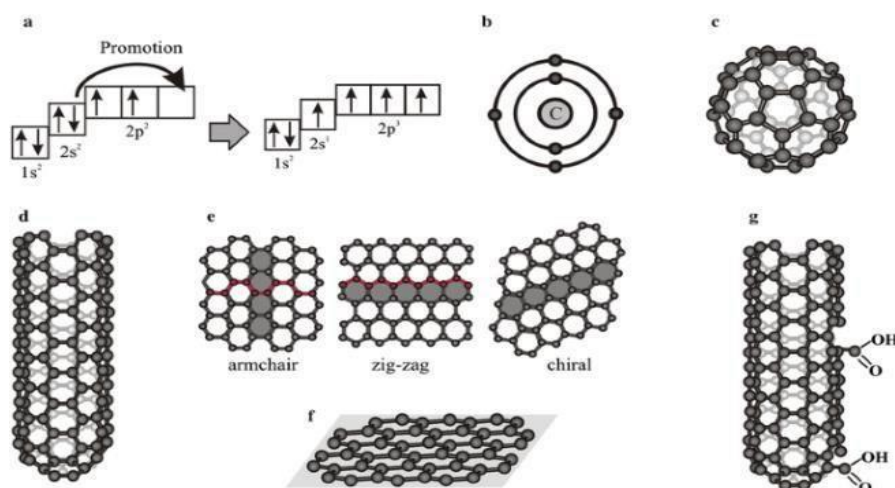


Fig. 2.2. Structure of a carbon atom and of carbon-based nanoparticles. a Electron configuration of a carbon atom before and after promotion of one s-electron; **b** schematic representation of a carbon atom structure with two electron orbitals around the nucleus and six electrons distributed on them; **c** structure of a fullerene C₆₀; **d** structure of a single-walled nanotube; **e** different types of single-walled nanotubes: *armchair*, *zig-zag* and *chiral*; **f** structure of a graphene sheet; **g** structure of an oxidized single-walled nanotube.

2.2.3 Graphene

Graphene is a 2D allotropic form of carbon, formed by single layers of carbon atoms (Fig. 2f). In graphene, carbon atoms exhibit sp^2 -hybridization combined by σ - and π -bonds in a two-dimensional hexagonal crystal lattice with a distance of 0.142 nm in the middle of neighboring atoms of carbon hexagons (Georgakilas *et al.*, 2015). Graphene also portrays a structural element of some other carbon allotropes, such as graphite, carbon nanotubes and fullerenes. Theoretical studies on graphene began ages before the real material samples were obtained. The Canadian theoretical physicist Wallace first inspected the theory of graphene in 1947, while the first graphene samples were described 57 years later (in 2004) by A. Geim (Dutch-British physicist) and K. Novoselov (Russian-British physicist), awarded with a nobel prize in the year 2010.

Despite the history of theoretical investigation, the fact that the real material has been obtained only recently implies that extensive studies on the properties of graphene are still ongoing. Graphene has various unique physical properties, such as extremely high mechanical rigidity and high thermal stability (Wen Su *et al.*, 2020). Also, the electric properties of this carbon allotrope fundamentally vary from the properties of three-dimensional materials.

2.3 Carbon Dots

CDs can be generally specified as a quasi-0D carbon-based material with a size lower than 20 nm, and fluorescence is their intrinsic property. In 2004, carbon nanoparticles with fluorescence were first announced, which were accidentally obtained from the purification of single-walled CNTs (Zhang *et al.*, 2014). In 2006, Sun and co-workers named nanoscale carbon particles synthesized by laser ablation of carbon selected as CDs for the first time, but the QY of these surface passivated CDs was only about 10% (Park *et al.*, 2014). Low QY and complicated preparation procedures limited the development of CDs (Mansuriya *et al.*, 2021). Until 2013, Yang's group chose citric acid (CA) and ethylenediamine as precursors and synthesized polymer-like CDs with QY up to 80% through one-step hydrothermal method (Hu *et al.*, 2009). This QY was the highest value for carbon-based fluorescent materials. These CDs can be applied both as printing inks and as functional nanocomposites. The facile proposal, high QY, low toxicity, and high

resistance to photo bleaching of CDs caused widespread concern and a research boom. Thereafter, researchers advanced different strategies and technologies to pursue CDs with high performance, and lots of significant breakthroughs have taken place in the past few years, including multicolor/deep red/near-infrared (NIR) emission narrow full width at half maximum (FWHM), two-multiphoton PL, chirality, room temperature phosphorescence (RTP), and thermally activated delayed fluorescence (TADF) as well as various applications (Kang *et al.*, 2012).

2.4 Classification Carbon Dots

At present, CDs are mainly classified into Graphene quantum dots (GQDs), Carbon quantum dots (CQDs) and Carbonized polymer dots (CPDs) according to their different formation mechanism, micro-/nanostructures, and properties, while associations can be built among them by changing the graphene layer and carbonization degree (Xia *et al.*, 2019).

2.5 Preparation of Carbon Nanomaterials

Fluorescent carbon nanoparticles were first isolated in 2004 by (Scrivens *et al.*, 2004) through the purification of single-walled carbon nanotubes. Therefore, various synthetic methods have been developed for the preparation of CDs with diverse sizes and surface functional groups. Typically, CDs can be synthesized by either top- down or bottom-up approaches. They are based on cutting the graphite matrix into nanosized particles and the chemical blending of small aromatic molecules, respectively (Yuan *et al.*, 2017). Applications of CDs in optoelectronic devices, the bottom-up approach is commonly regarded as an acceptable method for synthesizing bandgap fluorescence CDs. In the same, for biological applications, both methods are widely used for the preparation of CDs. Particularly, the top-down method can facilely deliver good water-soluble CDs for biological applications (Yuan *et al.*, 2017). The top-down method involves oxidative cleavage (Luo *et al.*, 2016) reductive cutting (Yuan *et al.*, 2019), physical grinding and laser ablation (Pan *et al.*, 2010).

2.5.1 Top-Down Approach

Top-down preparations of fluorescence CDs are the early preparation approaches, including arc- discharge method, electrochemical oxidation, and laser

ablation. In 2004, firstly separated accidentally fluorescent nanoparticles from the products when they prepared the single-walled carbon nanotubes by arc discharge (Xu, *et al.*, 2004). They also introduced nitril onto the fluorescent CDs by arc discharging fuming nitric acid, improving the hydrophilicity of nanomaterials. After that, NaOH solution (pH = 8.4) was used to extract the precipitate to give a black suspension and obtained three groups of fluorescent carbon nanoparticles with different molecular weights after further electrophoretic separation (Gonçalves *et al.*, 2010). These three groups of fluorescent carbon nanoparticles emit blue green, yellow, and orange fluorescence, respectively, wherein the fluorescence quantum yield of yellow phosphor carbon nanomaterials can reach 1.6%. Subsequently, Sun *et al.* pioneered in synthesis of fluorescent CDs by means of laser ablation.

Electrochemical methods for preparing fluorescent CDs. They used multiwalled carbon nanotubes as the working electrode grown in carbon membrane by means of chemical vapor deposition (CVD) method and blue fluorescence was obtained by cycle voltage (Zhou *et al.*, 2007). The size of the particle CDs produced in their method is 2.8 nm, and the fluorescence quantum yield is about 6.4%. Subsequently, some research groups obtained CDs by means of the same method only replacing the solution with aqueous solution and electrochemical oxidation of graphite rods shock. The fluorescence quantum yield is about 1.2%. In order to further improve the efficiency of the electrochemical method for preparing CDs, the researchers prepared the fluorescent CDs by using ionic liquid assisted electrochemical exfoliation of graphite electrodes. Using the ionic liquid solvent to replace the traditional solution can play a dual role of dissolving and catalysis, in which the reaction rate can be improved several times. Moreover, by adjusting the ratio of the ionic liquid and water, the fluorescence emission wavelengths of CDs can be adjusted. However, the fluorescence quantum yield of the CDs prepared in electrochemical methods is not high and needs further improvement. Compared with the multistep preparation method of CDs, one-step reaction processes not only simplify the preparation procedure and the prepared CDs also with better fluorescent properties, but also can obtain CDs with different fluorescent properties by choosing different organic reaction solution.

2.5.2. Bottom-Up Approach

Bottom-up approaches are focusing on the polymerization reaction for small

molecules to the formation of nanoscale CDs. This method includes hydrothermal method, microwave-assisted pyrolysis method, ultrasonic method, acid dehydration method, and pyrolysis method. The hydrothermal method is widely used among them and microwave-assisted pyrolysis method, which can be realized by the one-step method for preparing fluorescent CDs.

Liu *et al* (2011) prepared CDs by hydrothermal methods. They mixed candle ash as the carbon source with nitric acid through heat reflux and generated a black homogeneous solution mixture and then purified through a series of centrifugation, dialysis, gel electrophoresis, and other processes to obtain fluorescent CDs with different particle sizes. They identified that the emission wavelengths of fluorescent CDs depend on the sizes of particle even under the same excitation light (315 nm); the emission wavelength is moderately red shift with the increasing of the particle size, which reflects completely an optical character of fluorescent CDs, named elementary excitations and multi-emission (Tang *et al.*, 2014). A lot of reports about the research of preparing CDs through hydrothermal method using different carbon sources, and the fluorescence quantum yield of CDs has been greatly increased in the past two years. For instance, Gao and so forth chose C60 as the carbon source and CTAB as passivator to prepare CDs with high fluorescence QY up to 60%. Even more, the fluorescent CDs prepared in their method possess the distinctive property of aggregation induced enhanced emission, which differs from the common reports in the survey.

The microwave method is simple to synthesize the new type of CDs. With carbohydrate as a carbon source and PEG200 both as solvent and coating agent, the reaction system gradually changed from colorless to dark brown solution when kept under 500 W microwave power radiation for about 2~10 min, and the product obtained was diluted with water to obtain fluorescent CDs (Zhu *et al.*, 2009). In this method, the particle size and the quantum yield of fluorescent CDs depended on the reaction time. Wang *et al.* created a “green,” “fast,” “economy” CDs synthesis method by means of microwave method. The eggshell was first burned into ashes, then mixed the ashes with NaOH aqueous solution, and the solution was treated via microwave radiation for about 5 minutes to get CDs. The method is quite easy; the raw materials are cost effective and easily available, and less time consuming; the fluorescence quantum yield of CDs reaches 14%. (Chandra *et al.*,) used sucrose as

a carbon source and promoted the synthesis of fluorescent CDs in phosphoric acid environment by microwave radiation. The reaction time is just 3 minutes and 40 seconds, and the prepared CDs are shining bright in green fluorescence.

2.6 BIOLOGICAL APPLICATION

The physicochemical and optical properties of CQDs make them an outstanding candidate for biomedical applications. Their small size and cellular compatibility allow them to be used in bio-imaging, bio-sensing, and drug delivery vehicles that can be traced in the body using their fluorescence property. CQDs can be applied in various applications in the biomedical field such as gene delivery, electrochemical bio-sensing, pharmaceutical formulations, photodynamic and photothermal therapy, and treatment of bacterial infections and inflammations due to aforesaid properties of CQDs such as low toxicity, aqueous solubility, chemically inert, non-blinking, and excitation- and size-dependent PL emission.

2.7 BIOIMAGING

Bioimaging entails several techniques, which include positron emission tomography (PET) ultrasonic imaging, computed tomography imaging (CT), magnetic resonance imaging (MRI), optical imaging, etc. (Qu *et al.*, 2020). These techniques deliver images of biological materials, as well as their spatial and molecular details based on the type of required bioimaging application. Amidst these methods, fluorescent imaging has attained significant attention, because it is facile, affordable, and highly sensitive, as well as it generates high resolution photography (Koutsogiannis *et al.*, 2020; Zhang *et al.*, 2016). Assessing the bio compatibility of CDs before considering their biological application is important for any further advancement, which includes *in vitro* and *in vivo* tests. The *in vitro* toxicity is also known as the cytotoxicity while *in vivo* tests choose the whole organism as a research object. Until now, researchers have evaluated the *in vitro* cytotoxicity of various CDs. The *in vivo* toxicity of CDs is usually observed after injection of CDs. To date, there are few studies about the *in vivo* toxicity of CDs with different injection doses (Wang *et al.*, 2018). Therefore, bio-imaging techniques play a critical role in both fundamental research and clinical settings, allowing detailed observation of biological processes which considerably facilitates the intuitive study of cell morphology and cell physiological processes (Jhonsi *et al.*, 2018). In cancer

diagnostics, bioimaging is especially useful for the early detection of tumours and identification of metastasis as well as the resurgence of cancer. As a new class of bioimaging agents, CDs offer substantial advantages over inorganic semiconductor QDs and organic dyes in *in vitro* imaging owing to the unique combination of several key merits (Qu *et al.*, 2020). Therefore, CDs have been regarded as a sensitive biological probe for both *in vivo* and *in vitro* imaging (Qu *et al.*, 2020; Koutsogiannis *et al.*, 2020).

2.7.1 *In vitro* imaging

Given the significance of cancer research, most of the CD-based studies focus on cancer cell imaging, which was first reported in 2011 (Zh *et al.*, 2011). The work unveiled that GQDs with stable fluorescence emission hold promising potential in bio imaging (Anwar *et al.*, 2019). The attempts have been made for cancer cell imaging with various CDs, such as of MCF-7 cells, T47D cells, and HeLa cells, CDs with TP emission are urgently needed in cancer cell imaging, owing to the avoidance of harmful UV or blue excitation and the reduction of photo damage in bio-tissues. A facile approach was reported for the preparation of high-efficiency TP emission CDs with unique features of pure red emission ($\lambda_{\text{max}} \approx 640$ nm), high QY (22.9%), low cytotoxicity, high photo thermal conversion efficiency (43.9% under irradiation with a 671 nm laser) and TP excited fluorescence, These red emissive CDs (R-CDs) were verified to be capable of TP fluorescence bioimaging in living cells (Fig. 2.3). As seen in TP mode, red emission from MCF-7 and HeLa cells can be observed under irradiation with an 850 nm fs pulse laser, clearly indicating the abilities of the R-CDs as TP bioimaging agents. Quantification of the fluorescence intensity profiles unveiled the stronger emission in the nucleoli regions, indicating that the R-CDs were capable of nucleolus staining. Because of the nucleolus being an RNA-rich region, the observed nucleolus staining implies that the R-CDs have higher affinity to RNA than DNA and other substances in the cytoplasm. Besides, the TP absorption cross-section is an important evaluation parameter for evaluating the luminescence efficiency. Nitrogen-doped GQDs (N-GQDs) have been used as efficient TP fluorescent probes for HeLa cell imaging. The HeLa cells became illuminated when imaged under irradiation at 800 nm. The TP absorption cross-section of the N-GQDs reached 48 000 Goppert – Mayer units, which far surpassed that of organic dyes and was comparable to that of high-performance semiconductor QDs.

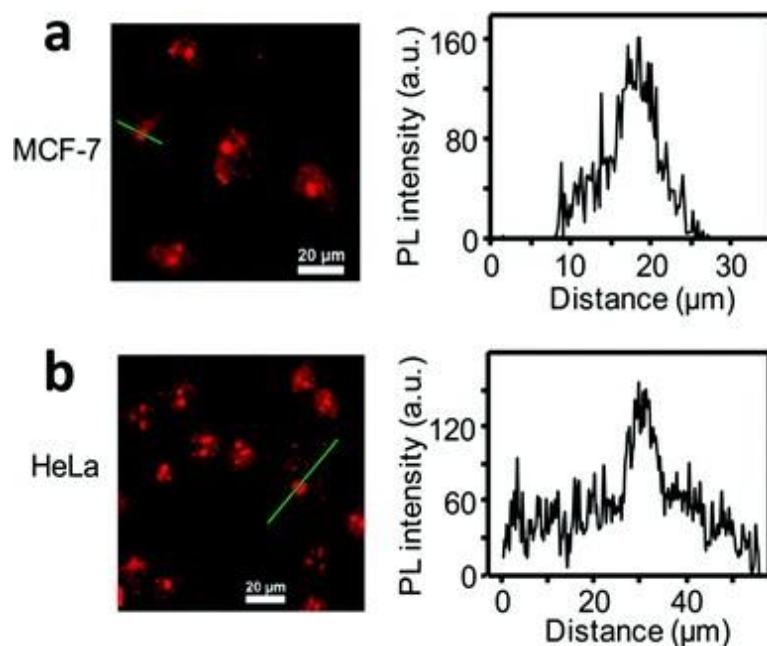


Fig. 2.3 TP confocal fluorescence images of (a) living MCF-7 cells and (b) living HeLa cells, and corresponding quantitative figure along the green line ($\lambda_{\text{ex}} = 850 \text{ nm}$ fs pulse laser, $\lambda_{\text{em}} = 600\text{--}700 \text{ nm}$, scalebar=20μm).

2.7.2 *In vivo* imaging

Apart from *in vitro* cell imaging, CDs are increasingly used for *in vivo* cell imaging and have provided fruitful results. Afterwards, a variety of CDs have been synthesized and used for *in vivo* imaging. Excitation with longer wavelengths is highly preferred for *in vivo* imaging due to deeper light penetration into the specimens. However, most previously reported CDs emitted only blue to green fluorescence. CDs for *in vivo* imaging emitting in the red or NIR region are still challenging. Tumour selective imaging *in vivo* regardless of the origin and location but not in normal tissues is also a major task. The fluorescence intensity of IR780/GQD-FA accumulated in the tumour areas and peaked at 24 h post-injection. These are fluorescence imaging agents for cancer diagnosis in living systems. Mice were sacrificed 24 h post-injection, and the excised tumour exhibited strong fluorescence intensity, whereas the normal organs revealed very low signals, which was better than the *in vivo* behavior of many other previously reported CDs.

2.7.3 Biosensors

Biosensors have been widely applied for analyzing small biological molecules, metal ions and proteins. In the past few years, inorganic semiconductor-

based biosensors have been broadly investigated in numerous studies, exhibiting high fluorescence QY and photo stability (Wen Su *et al.*, 2020). CQDs are also used as biosensors of their aqueous solubility, non-toxic nature, excellent cellular compatibility, excitation-dependent multicolor emission, permeability to cells, high photostability, and low toxicity. The CQD-based biosensors can visually monitor glucose (Shi *et al.*, 2011), phosphate (Zhao *et al.*, 2011), nucleic acid (Li *et al.*, 2011c), cellular iron (Zhu *et al.*, 2012), potassium iron, and pH (Wei *et al.*, 2012). CQDs are used as an excellent fluorescent assay to detect nucleic acid with selective single-base mismatch. CQDs adsorb *via* p–p interactions to label a single-stranded DNA (ssDNA) probe, accompanied by fluorescence quenching and hybridizing to its target to form double-stranded DNA (dsDNA), resulting in desorption of dsDNA from the CQD surface with more recovered fluorescence, hence probing the target DNA (Li *et al.*, 2011). Reactive oxygen species (ROS) are important biomarkers for many diseases including cancer, DNA damage, infections or inflammations, arthritis, neurodegenerative disorders, and chemotherapeutic drug screening. Manipulation of reactive oxygen species (ROS) using oxidation–reduction active substances is a means to kill cancer cells selectively. ROS sensors have been developed to detect reactive oxygen species (ROS) using encapsulated CQDs in hydrogel made of ascorbic acid. They are used to check the efficacy of the drug by measuring ROS levels after administering chemotherapeutic compounds (Bhattacharya *et al.*, 2017). N-doped CQDs synthesized from the one-pot pyrolysis method with a high QY of about 49.5% and high photoluminescence are used in the detection of β -glucuronidase (GLU) inhibitor activity based on the inner filter effect (IFE). GLU used as a biomarker in early-stage cancer diagnosis and many physiological diseases has a great importance as a biomarker because of its inhibition activity to control the proliferation of cancer cells with the ability to change the fluorescence intensity of N-doped CQDs (Lu *et al.*, 2016).

CQDs synthesized by the electrochemical method of average size 6 nm were used to develop a two-photon fluorescent probe for observing pH changes from 6 to 8.5 with high sensitivity and specificity. The described probe was effectively used in two-photon biosensing and bioimaging to monitor the pH changes in living cells

and tissues. Kong and his colleagues demonstrated that the terpyridine-based receptor molecule (TPY) covalently binds to the CQD surface and induces change in fluorescence intensity due to adsorption–desorption of H^+ ions. The quantum yield of CQDs was approximately 10% and showed CQDs possess UCPL characteristics from UV to NIR spectra. The CQD-TPY nano-probe shows high selectivity toward H^+ ions as compared to other metal atoms, biomolecules, and amino acids, as the H^+ ion concentration increases, and the emission intensity also increases in the 440–650 nm range. The imaging and biosensing of pH in real time were successfully accomplished in *in vitro* lung cancer A549 cells and mouse LLC- MK2 cells and *in vivo* tumor tissues by inducing tumor cells in nude mice through two-photon microscopy (Kong *et al.*, 2012).

So far, multiple studies have shown that CQDs fabricated by facile methods can show selective and sensitive detection of various ions, metals, and molecules. The ability of CQDs to detect metal ions is the inevitable sensing application including biological and chemical scenarios. This is due to attraction and alignment of metal ions with aromatic residues present on the surface of CQDs. Even at low concentrations when metal binds to CQDs, they cause luminescence quenching; hence, they are an effective vehicle for metal sensing. CQDs were prepared from N- (β -aminoethyl)- γ -aminopropyl methyldimethoxysilane (AEAPMS), and the residues ethylenediamine and methoxysilane groups were on the surface to detect Cu^{2+} ions and were coated on rhodamine B–doped silica nanoparticles (Liu *et al.*, 2014). Under single excitation wavelength, rhodamine B emitted red fluorescence and CQDs emitted blue fluorescence. When CQDs interacted with Cu^{2+} ions, they quenched fluorescence without affecting rhodamine B. The *in vitro* test was conducted to investigate the feasibility of dual emission nanoparticles by incubating them with MCF-7 cells. Blue and red wavelengths were emitted, and fluorescence images were taken. When Cu^{2+} ions were added, fluorescence intensity of blue decreased more as compared to that of red. All these findings suggest that CQDs can act as non-invasive detectors that are inexpensive and sensitive and help in improving human health.

2.7.4 Drug Delivery

While cancer and other diseases are being conventionally treated by chemotherapy, such a route typically lacks precision and causes problems of toxicity and multi-drug resistance (Li X. *et al.*, 2014). Therefore, targeted drug delivery is preferred as an alternative method to improve drug bioavailability and efficacy and reduce side effects (Sharma *et al.*, 2006; Rosenholm *et al.*, 2010; Liu *et al.*, 2012). But it also causes the drug to leak before it reaches the target site (Zhou *et al.*, 2014). Hence, efficient target agents like CQDs are required as they have been proposed as dual nano-carrier systems comprising of bioimaging and specific drug delivery agents with minimum cytotoxicity. CQDs modified through functionalization avoid cytotoxicity through drug leakage to normal cells. Functionalized CQDs contain amino groups on their surfaces which are used to crosslink with tumor theranostics. CQDs prepared from citric acid through conjugated with theranostic doxorubicin (DOX) through acid-labile Schiff base linkage. The *in vitro* test showed that theranostic nanoparticles had the ability to release drugs without any burst release in the acidic microenvironment of the tumor in a pH-dependent manner. Moreover, their fluorescence property provided imaging-guided drug delivery (Jia *et al.*, 2016).

CQDs have also been used for targeted delivery in the nucleus and mitochondria (Wu *et al.*, 2017). Mitochondria plays a vital role in many cancers and metabolic disorders. CQDs produced from chitosan, ethylenediamine, and mercaptosuccinic acid *via* hydrothermal treatment were found to possess long-term mitochondrial imaging as compared to commercially available Mito-Tracker dye and mitochondrial-specific temperature-dependent transport and receptor-mediated endocytosis (Hua *et al.*, 2017).

Fluorescence of CQDs and location of their emission help to differentiate the living and apoptotic cancer cells. CQDs incorporated in hydrogel were taken up by A549 cells and emitted green light when observed under fluorescence microscopy. The emission of green light depends upon the concentration of CQDs in the gel. Hydro thermally synthesized CQDs have been used in the drug delivery system to deliver the anticancer drug (DOX) to the targeted area (Molaei *et al.*, 2019).

In another study, it was observed that drug loaded CQDs were more localized as compared to DOX alone. To check their activity in the body, conjugated CQDs were more cytotoxic in cancer cells as compared to normal cells (Lee *et al.*, 2014; Nayab Azam *et al.*, 2021).

2.7.5 Gene Delivery

Gene therapy has significant importance in the field of biotechnology and medical science applications, focusing on the treatment of diseases and correction of expression of genes on DNA, encoded for missing or defective genes. Usually, a tumor-suppressor gene is incorporated into the tumor cells inhibiting development in gene therapy or gene delivery. Nevertheless, nanomaterials like CDs have proven systematic application as nanocarriers/gene carriers in gene delivery or gene therapy, owing to their outstanding biocompatibility and non-toxicity. Therefore, various types of nanoparticles are used in gene therapy to distribute genes at the selected sites of DNA. In a study, theranostic and reducible nano-agents' fc-rPEI-CQDs were developed and characterized. These nano-agents are extremely biocompatible and are excellent gene delivery carriers for the treatment of targeted lung cancer by encapsulating siRNAs and discharge them in a reductive environment (Wu *et al.*, 2016). Cationic dual-function CQDs produced by the one-step microwave pyrolysis method have been used as self-imaging and non-viral gene delivery agents in chondrogenesis from fibroblasts. These cationic CQDs have beneficial properties such as tunable fluorescence, lower cytotoxicity, biocompatibility, and huge quantum yield. Cationic CQDs can condense the gene vector SOX9 to cast a very small nanostructure having a size of 10–30 nm for their application (Cao *et al.*, 2018).

CQDs can be utilized as a gene carrier. According to research, PCD and DNA were mixed to form the PCD/DNA compound. By agarose gel electrophoresis, the potential of CQDs to condense DNA was observed. Enhanced green fluorescent protein (EGFP) was applied as a gene to visualize the *in vivo* gene expression. Emission of green fluorescence demonstrates the expression and delivery of genes into the selected site. PCD emitting blue fluorescence specifies that PCD could be

used as a tag for gene delivery and bioimaging because of this bright blue fluorescence and high transfection ability (Hu *et al.*, 2014). In another study, polyethylenimine- functionalized CQDs (CQDs-PEI) appeared multicolor fluorescence having the ability of both gene delivery and bioimaging. CQD-PEI/DNA complexes were used for the transmission and labeling of COS-7 cells. After transfection, COS-7 cells had strong fluorescence emitted from CQDs-PEI dispersed in the cytosol. Passivated CQDs have the capacity to emit different colors such as blue, green, and red at different excitation wavelengths and may have the future to be used in *in vivo* fluorescence tracking (Liu *et al.*, 2012).

CHAPTER 3

AIM AND SCOPE

3.1 AIM OF THE PROJECT

The aim of the project is that:

- To prepare and characterize carbon nanodots using red alga *Kappaphycus alvarezii* and their bio-imaging applications.

3.2 SCOPE OF THE PROJECT

The scope of the project:

- Green methodologies for the preparation of luminescent carbon dots and their bio-imaging application.

CHAPTER 4

MATERIALS AND METHODOLOGY

4.1 COLLECTION AND PREPARATION OF SEAWEED EXTRACT

Seaweed *Kappaphycus alvarezii* (Fig. 4.1) was collected from Mandapam camp (9.2815° N; 79.1376° E), Southeast Coast of Tamil Nadu, India. The identification of seaweed was done by previously described standard keys (Sahoo *et al.*, 2010). The seaweed samples were thoroughly washed with distilled water to remove the associated biota, salt and other debris. Further, the seaweed samples were dried and powdered using an electric blender. One gram of powdered seaweed was mixed with 100 ml of deionized water and the solution was kept for 24 h while being gently suspended by a magnetic stirrer. The procedure was completed by filtration seaweed extract that was further used for the preparation of carbon dots.



4.1 Seaweed *Kappaphycus alvarezii*

4.2 PREPARATION OF CARBON DOTS

Seaweed extract (45 ml) was mixed with 5 ml of methanol, then the extract was placed in the 100 ml Teflon-coated autoclave, sealed, and incubated in the muffle furnace at 120 °C for 3 h. After, the solution allowed to at ambient room temperature. Further, the solution was centrifuged at 5,000 × *g* for 30 min to remove the unwanted particles/debris. The supernatant was collected for further characterization.

4.3 CHARACTERIZATION OF CARBON DOTS

4.3.1 UV-VISIBLE SPECTROSCOPIC ANALYSIS

UV-visible spectroscopy is used to analyze the absorption characteristics of the carbon dots using Shimadzu UV-1800, Japan.

4.3.2 X-RAY DIFFRACTION (XRD) ANALYSIS

To investigate the structural and phase analysis of the prepared carbon dots using XRD analysis was carried out using Rigaku MiniFlex 600 Desktop X-Ray Diffraction System.

4.3.3 PHOTOLUMINESCENCE (PL) STUDIES

Photoluminescence (PL) spectra was used to determine the excitation and emission spectra of the carbon dots using Shimadzu-RF5301pc, Japan.

4.3.4 FOURIER TRANSFORM INFRARED (FT-IR) SPECTROSCOPY STUDIES

To determine the functional groups, present in the carbon dots solution using Fourier- transform infrared spectroscopy (FTIR, Bruker Germany).

4.3.5 TRANSMISSION ELECTRON MICROSCOPY STUDIES

The size and shape of the carbon nano-dots were examined using Transmission electron microscopy (TEM-JEOL 3010).

4.3.6 PARTICLE SIZE MEASUREMENTS

DLS measurements (particles size) were carried out using Horiba SZ100, Japan

4.4 CONJUGATION OF DRUG WITH CARBON DOTS

Drug loading Equal volumes of 0.5 mg/ml of Penicillin/Streptomycin and carbon dots were mixed in distilled water with stirring (300 rpm, 27°C) for 3 h in the absence of light. Unbound Penicillin/Streptomycin was removed from the mixture by centrifugation with Vivaspin centrifuge filter cartridges with the molecular cut-off 5 kDa for 20 min at $5000 \times g$ to obtain the conjugated carbon dots bound to Penicillin/Streptomycin. A UV-vis Spectrophotometer (Shimadzu UV-1800, Japan) was used to measure the loading capacity of carbon dots, by comparing the carbon

dots-drug (Penicillin/Streptomycin) absorption peak with the Penicillin/Streptomycin standard curve at absorbance peak of Penicillin/Streptomycin. The drug loading was calculated according to the following equation:

$$\text{Drug loading (\%)} = \frac{\text{Mass of Penicillin/Streptomycin in carbon dots}}{\text{Mass of Penicillin/Streptomycin in feed}} \times 100$$

4.5 Bacterial Cell bio-imaging assay

E. coli and *S. aureus* cells were grown in Luria Bertani (LB) medium. The cultures were shaken at 150 rpm, and growth was monitored by measuring the absorbance at 600 nm. *E. coli* and *S. aureus* cells were collected from the middle of the exponential growth phase and used for fluorescence microscopy analysis. For carbon dots internalization studies, cells were fixed, and the fixation was performed with 70 % (v/v) ethanol at 4 °C for 5 min. The fixed cells were re-suspended in phosphate buffer (100 mM) containing carbon dots at room temperature for 10 min. The stained bacterial cells were washed twice and subjected to fluorescence microscopy. Cell imaging was done under a Carl Zeiss Axioscope microscope with UV region.

CHAPTER 5

RESULTS AND DISCUSSION

Carbon quantum dots have proven to be effective in drug delivery mostly owing to their essential properties of biocompatibility and non-bleaching photoluminescence, enabling high cargo transport and long-term imaging and tracking.

5.1 Preparation of carbon dots

The luminescent carbon dots were prepared by using seaweed as carbon source material, nucleation, and growth conditions. The carbon source material and conditions play a key role for the preparation of carbon dots with tunable emission and with high quantum yield. The color of seaweed extract was changed from brown to dark brown after heating the seaweed extract at different temperatures 120 °C, and 140 °C for 180 min, among which 140 °C confirming the formation of carbon dots solution. It can be observed that the CDs showed intense bright blue color emission only at UV light excitation wavelength 365 nm (Fig. 5.1a&b).

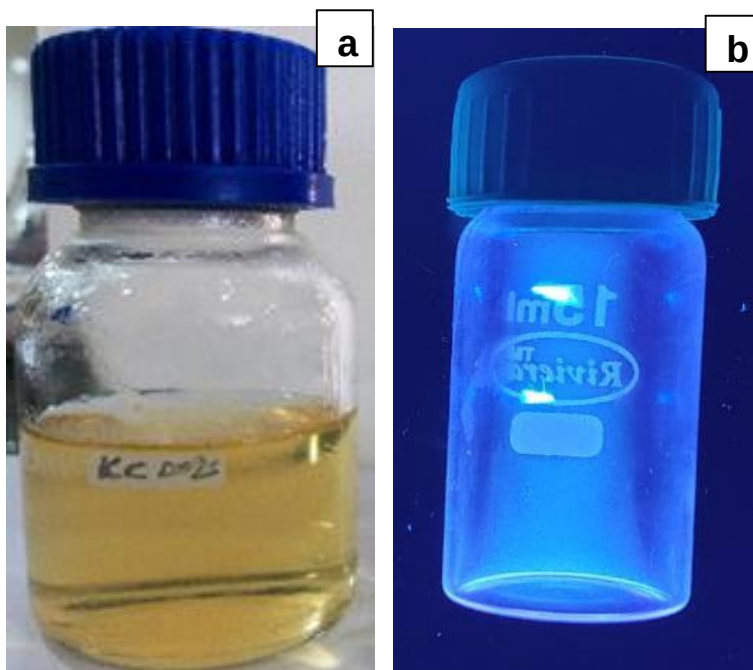


Fig 5.1. Photographic images of synthesized carbon dots at 140 °C (a) and their fluorescence properties under UV light excitation at 365 nm.

5.2. UV-vis spectroscopic studies

Fig. 5.2 depicts the UV-Vis absorption spectrum of carbon dots solution. The carbon dots show the UV absorption peak at 273 nm, which is the characteristic peak for carbon dots by carbonization of renewable carbon source. This absorption peak confirms the carbon π system or the $n-p^*$ transition of the carbonyl functional group. This result is in consistent with the previous reports (Roshni *et al.*, 2015; Sahu *et al.*, 2012; Shereema *et al.*, 2015; Hoan *et al.*, 2019).

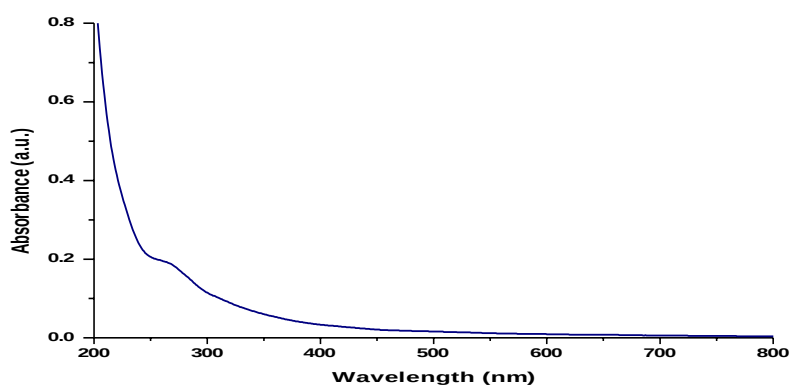


Fig. 5.2. UV-visible absorption spectra of carbon dots synthesized using seaweed

5.3. Photoluminescence spectroscopic studies

Photoluminescence spectrum of carbon dots which was recorded at an excitation wavelength, $\lambda_{\text{ex}} = 330$ nm (Fig. 5.3). From the photoluminescence spectrum, the broad emission band was observed at around 400 nm. This blue emission is mainly considered to be the versatile characteristic of carbon dots, which may be due to quantum confinement effects, small size distribution or the presence of emissive traps on the surface (Roshni *et al.*, 2015; Park *et al.*, 2014; Sahu *et al.*, 2012; Cao *et al.*, 2007).

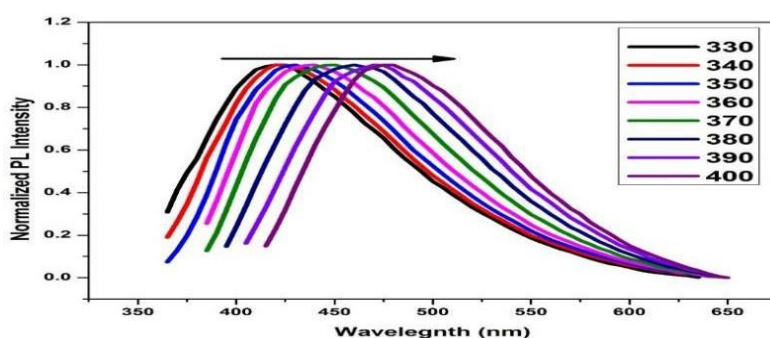


Fig. 5.3. Photoluminescence spectrum of carbon dots synthesized using seaweed

5.4. FT-IR spectroscopic studies

FT-IR analysis is mainly utilized to study the functional groups which are present in the prepared carbon dots. Fig. 5.4 shows the FT-IR spectrum of carbon dots. From the spectrum, the characteristic absorption broad band is observed at around 3330 cm^{-1} which is mainly due to O-H stretching vibration of hydroxyl group. Two sharp peaks were detected at around 1642 and 1020 cm^{-1} which corresponds to aromatic C=C (ring stretching) and CO stretching of alcoholic OH or C-O-C group (Roshni *et al.*, 2015; Sahu *et al.*, 2012; Shereema *et al.*, 2015).

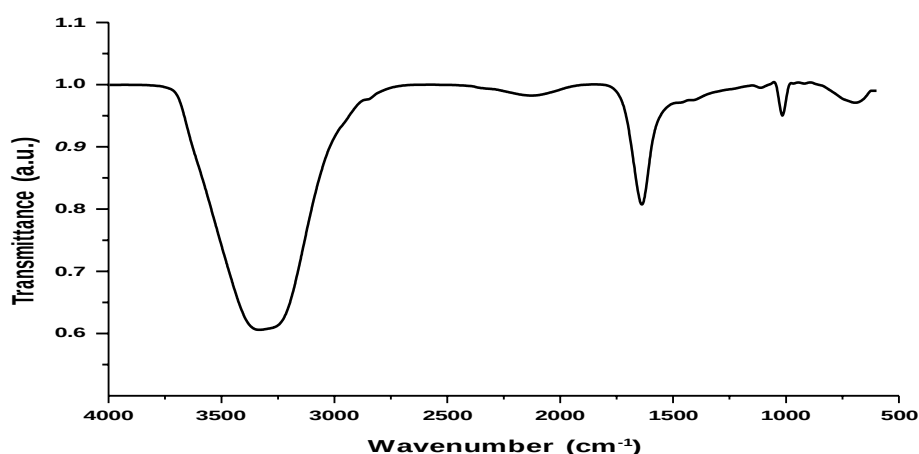


Fig. 5.4 shows the FT-IR spectrum of carbon dots synthesized using seaweed

5.5 TEM and DLS analysis

In order to investigate the morphology and average size of synthesized carbon dots and study were characterized using TEM and DLS. Fig. 5.5a&b shows the typical TEM images of carbon dots in which most of carbon dots are well dispersed in aqueous solution with uniform in size and possessed a nearly spherical shape with an average size of $\sim 12\text{ nm}$. Fig. 5.5c shows the DLS data of carbon dots, in which carbon dots are well mono-dispersed with an average hydrodynamic diameter of $\sim 15.25\text{ nm}$. These data are well agreed with the above TEM images.

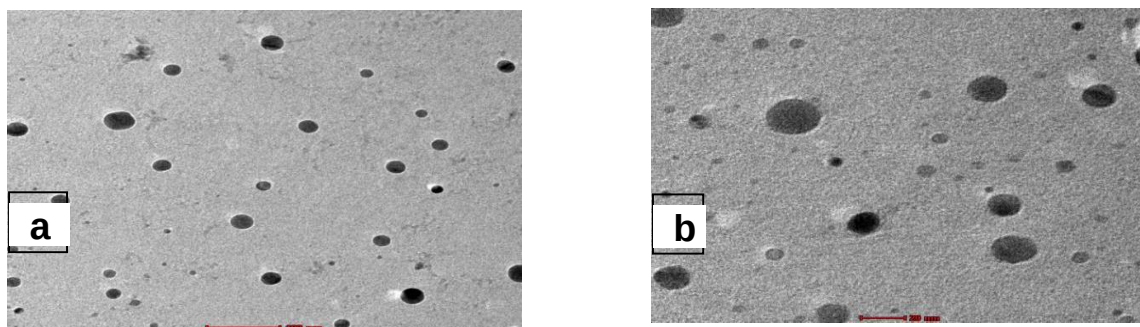


Fig. 5.5 a&b shows the TEM images of carbon dots synthesized using seaweed

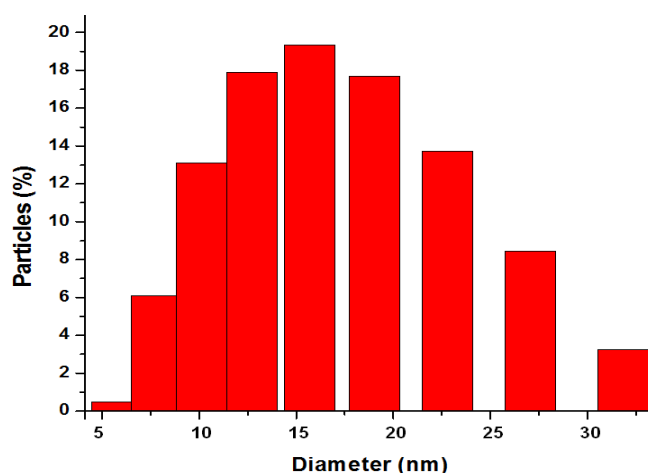


Fig. 5.5c DLS measurement of carbon dots in aqueous solution

5.6. XRD studies

The carbon dots were examined using X-ray diffraction. An X-ray diffractometer was utilized to produce patterns using the drop cast method. As shown in Fig. 5.6 the carbon dots have exhibited the intense peak at $2\theta = 28.72^\circ$ (002) and a weak peak at $2\theta = 41.63^\circ$ (101), confirming the diffraction patterns of graphitic carbon in seaweed extract, which is strongly agreed with reported methods (Pandey *et al.*, 2013; Liang *et al.*, 2013; Jia *et al.*, 2012).

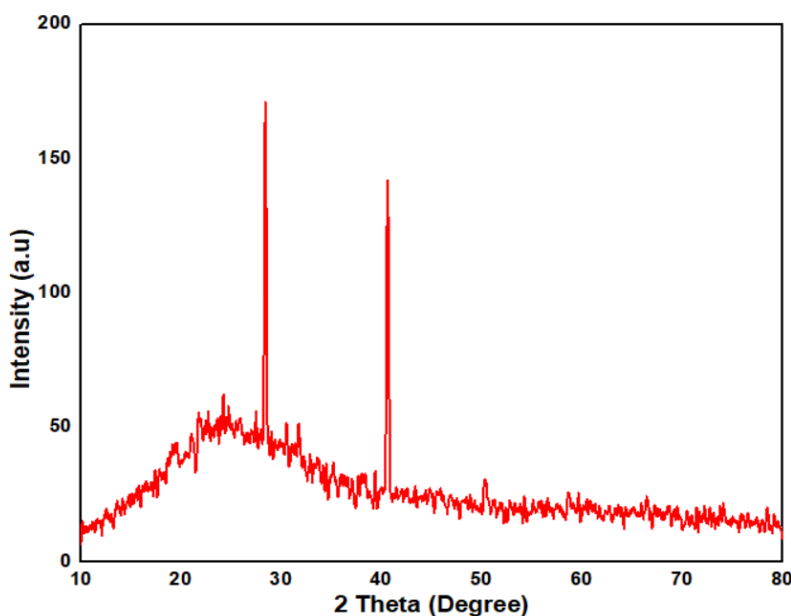
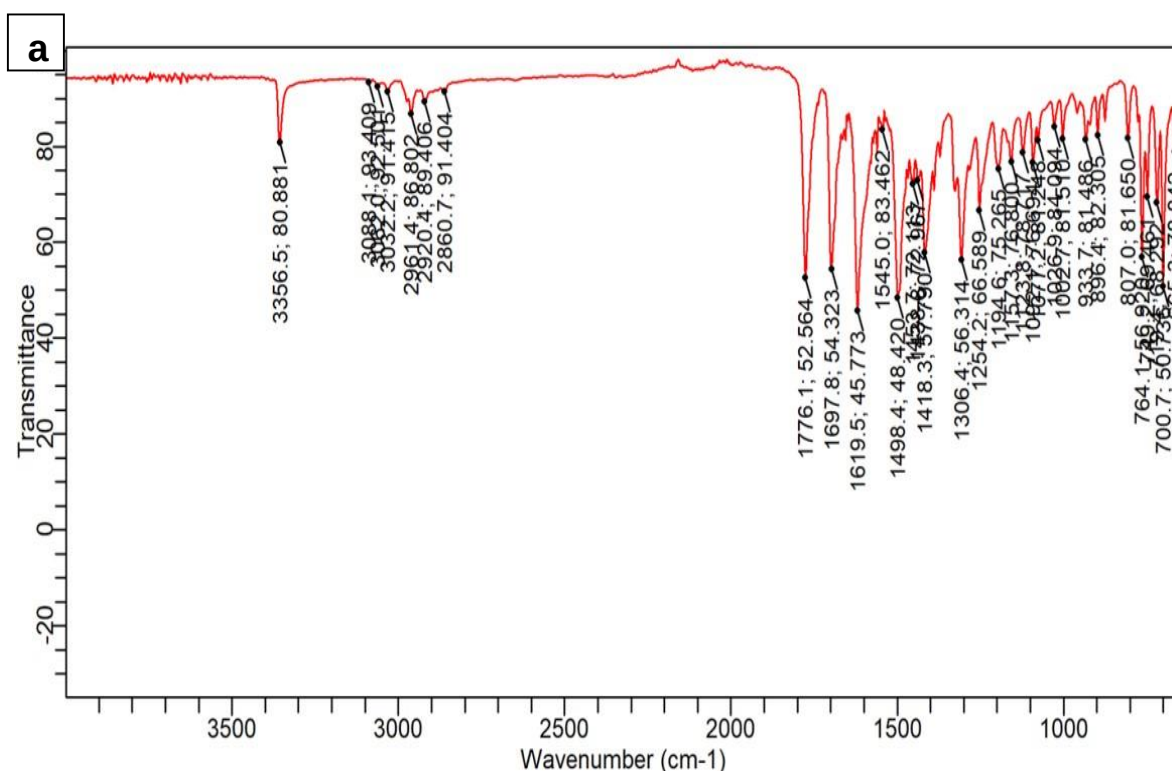
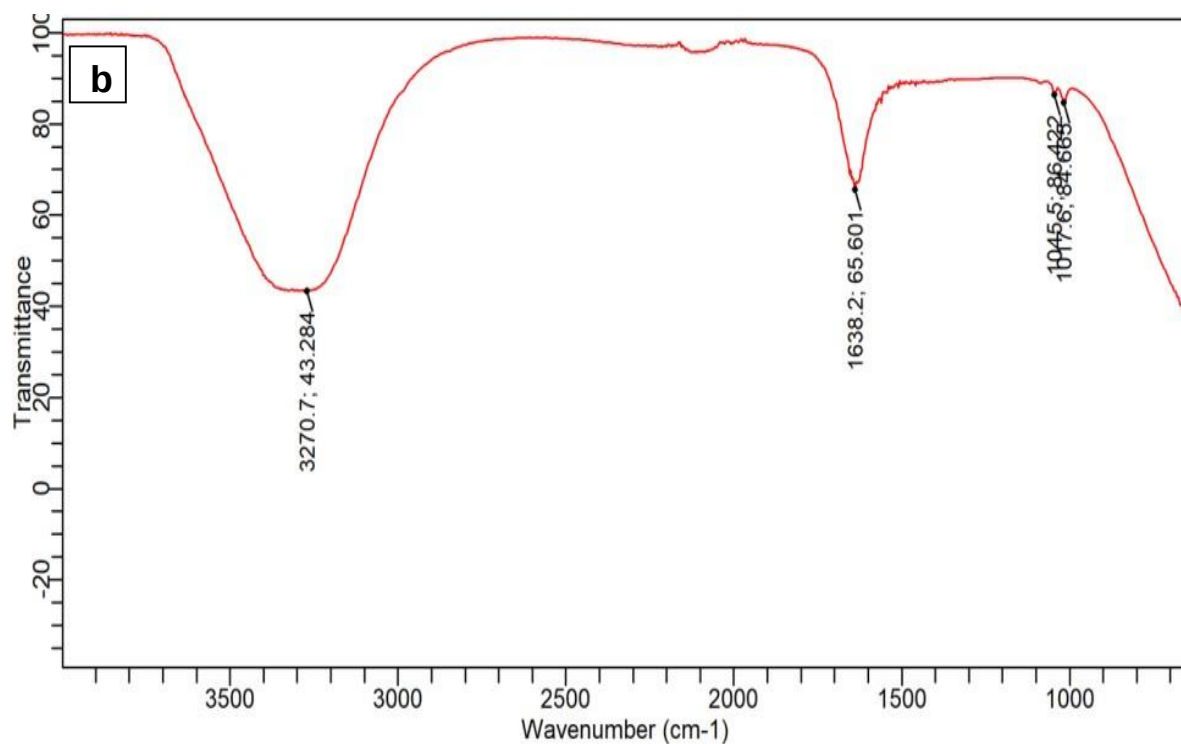


Fig. 5.6 XRD pattern of carbon dots in aqueous solution

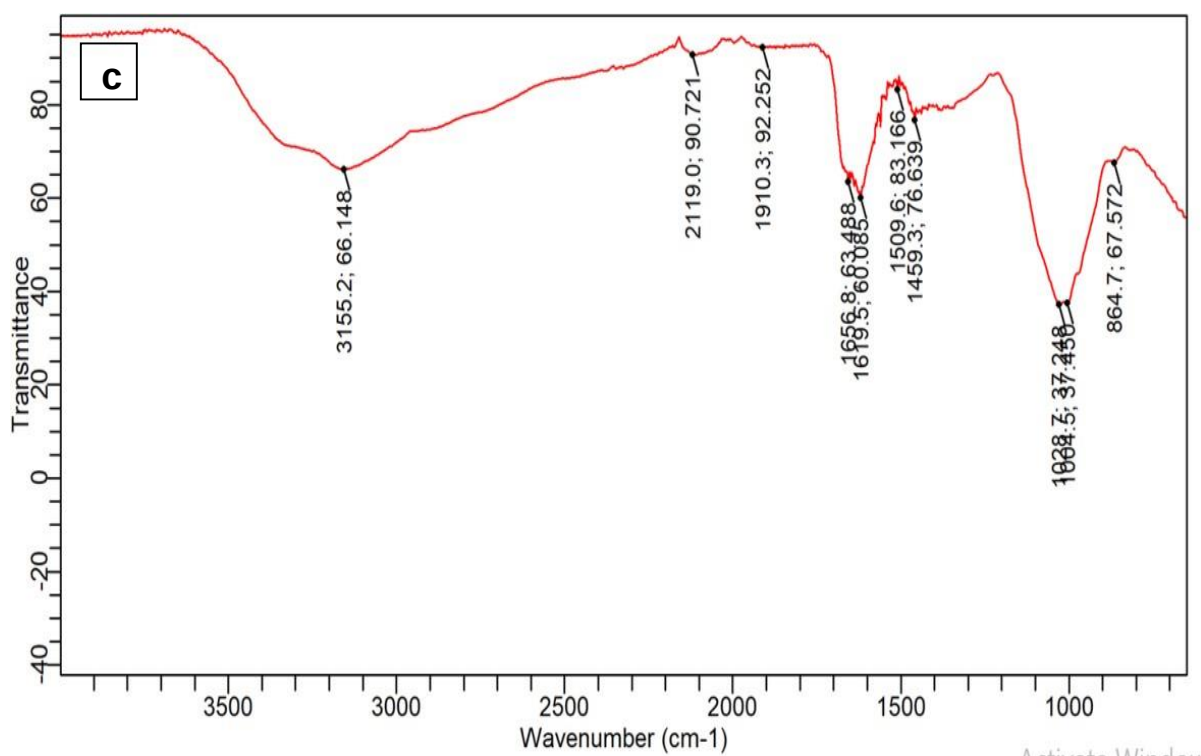
5.7. Drug loading efficiency

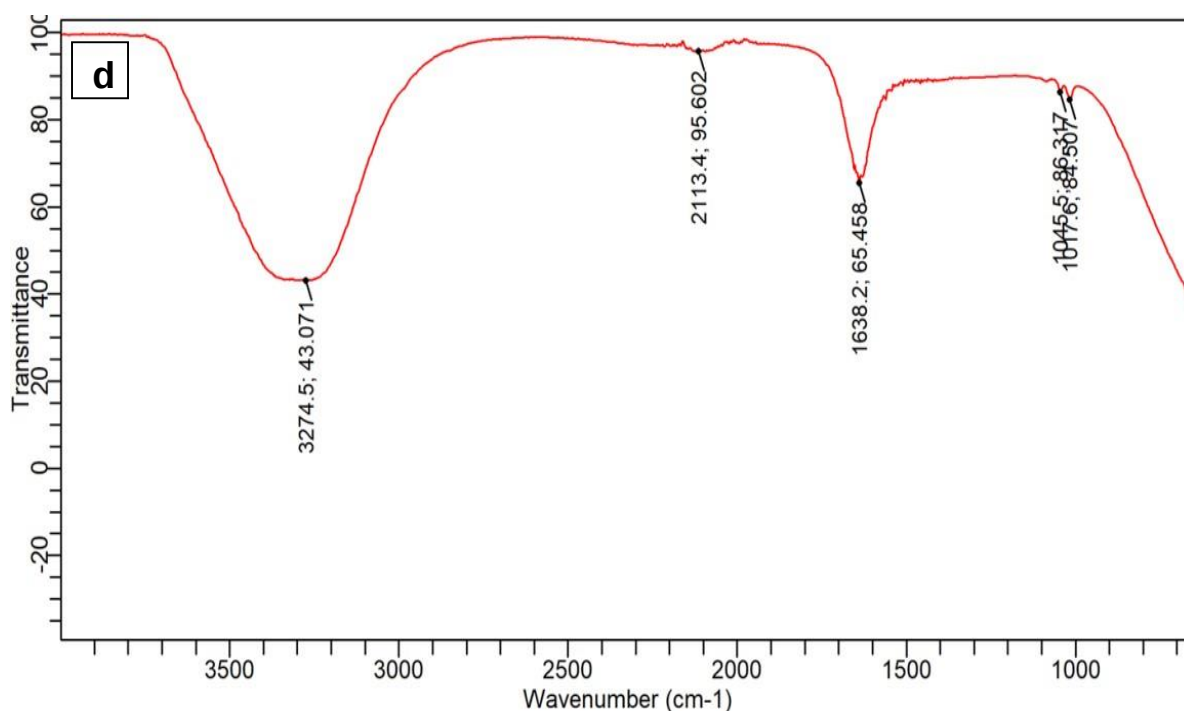
The antibiotics penicillin and streptomycin were conjugated onto carbon dots, and the drug loading capacity was found to be 83% (w/w) using UV–Vis spectroscopy. Based on the penicillin/streptomycin and carbon dots molecular weights and relative drug loading capacity, the average molar ratio of carbon dots to penicillin/streptomycin in the penicillin/streptomycin-carbon dots were calculated to be 1:75. The Fischer esterification reaction and hydrogen bonding were confirmed to be the primary mechanisms of drug loading using FTIR spectroscopy. In summary, the carboxylic acid moiety of carbon dots reacted with the alcohol group on the penicillin/streptomycin to form an ester. As can be seen in Fig. 5.7a-d, the Fischer esterification reaction is clear at 1600 cm⁻¹ (contributed by ester C=O stretch) with the slight broadening of the peak, accommodating both C=O and ester bonds at the same wavenumber. Further, also note the presence of hydrogen bonding at around 3200 to 3550 cm⁻¹.





5.7. FTIR spectra confirming drug loading via esterification and hydrogen bonding
(a) Penicillin and (b) carbon dots-Penicillin after conjugation.





5.7. FTIR spectra confirming drug loading via esterification and hydrogen bonding
 (c) Streptomycin and (b) carbon dots- Streptomycin after conjugation.

5.8. Bacterial cellular uptake studies using drug-carbon dots

Nanobiotechnologists have found ways to visualize cells in microorganisms by using carbon dots as fluorescent imaging probes. The carbon dots are fluorescent nanometer-scale crystals that consist of an electron-dense core composed of carbon in order to facilitate bio-conjugation. The carbon dots are becoming increasingly popular as cellular probes for light microscopic imaging because of their unique optical and physical properties. These include high fluorescent quantum yield, resistance to photo-bleaching, a large absorption cross section, and the ability to precisely tune the fluorescence emission. To extend the application potential of carbon dots as fluorescent imaging probes, we have investigated the carbon dots as *in vivo* cell imaging probes for *Escherichia coli* and *Staphylococcus aureus*.

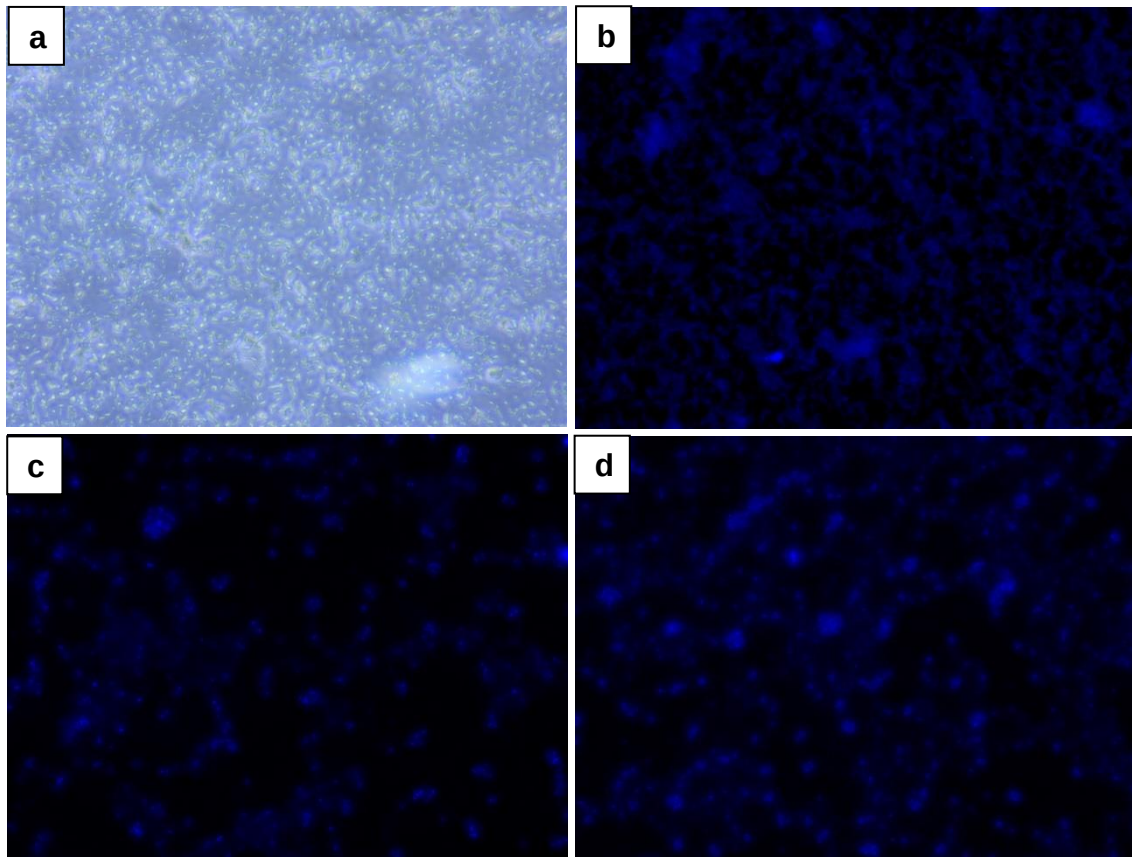


Fig 5.8 shows a) bright field image of *Escherichia coli* cells; b) fluorescence microscopic image of carbon dots treated *Escherichia coli* cells; c) penicillin conjugated-carbon dots treated *Escherichia coli* cells; d) streptomycin conjugated-carbon dots treated *Escherichia coli* cells.

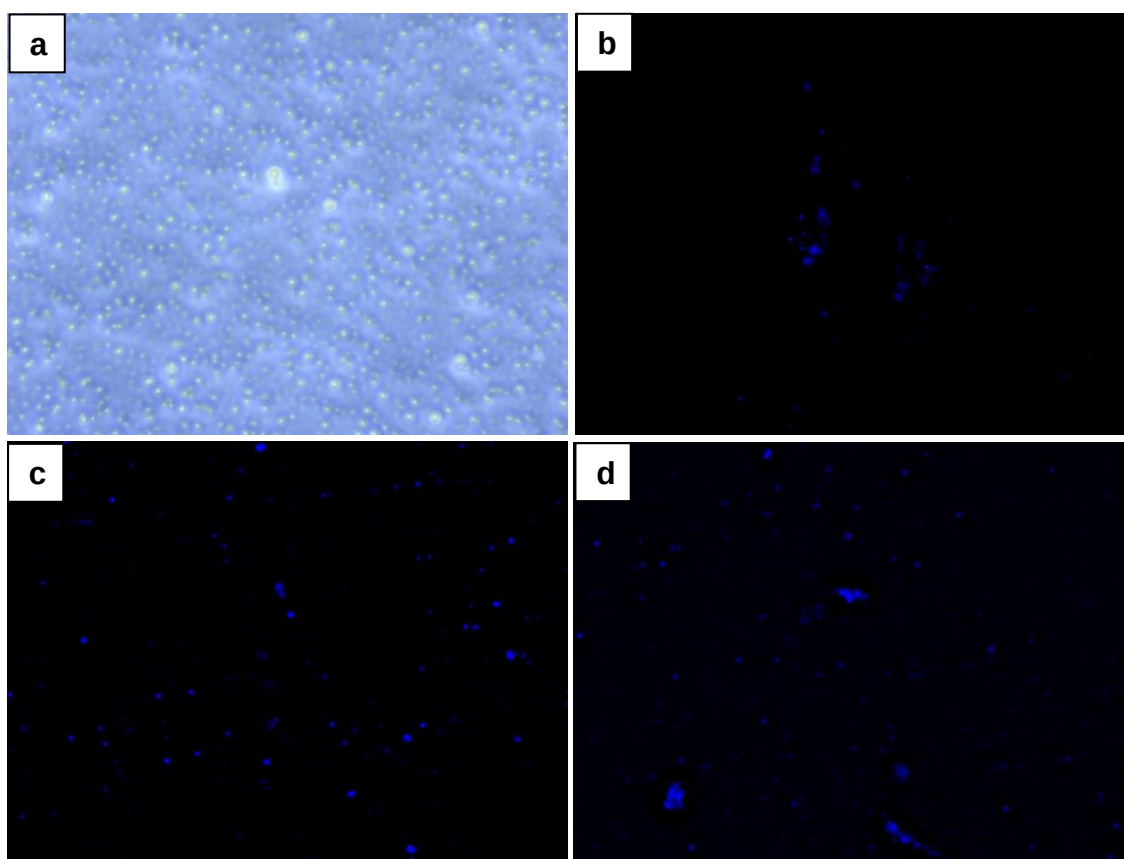


Fig 5.9 shows a) bright field image of *Staphylococcus aureus* cells; b) fluorescence microscopic image of carbon dots treated *Staphylococcus aureus* cells; c) penicillin conjugated-carbon dots treated *Staphylococcus aureus* cells; d) streptomycin conjugated-carbon dots treated *Staphylococcus aureus* cells.

Fig. 5.8a & 5.9a shows the bright filed images *Escherichia coli* and *Staphylococcus aureus* cells. Fig. 5.8b and 5.9b show the fluorescence microscopic images of carbon dots treated *Escherichia coli* and *Staphylococcus aureus* cells. 5.8c & 5.9c show the fluorescence microscopic images of penicillin conjugated-carbon dots treated *Escherichia coli* and *Staphylococcus aureus* cells. 5.8d & 5.9d show the fluorescence microscopic images of streptomycin conjugated-carbon dots treated *Escherichia coli* and *Staphylococcus aureus* cells. These results illustrate that the carbon dots and penicillin/streptomycin conjugated carbon dots were effectively up taken to the cells, which results in a possible endocytosis mechanism through their homogeneous distributions inside the cell (Hsu and Chang, 2012). Further, the results successfully illustrate that carbon dots possess good biocompatibility and can be effectively applied multiple fluorescence emission for *in vitro/in vivo* cell imaging and biological labeling. Importantly, it has been observed that these results are quite different from the report of CdSe quantum dots as fluorescent imaging probes for yeasts

(Bao et al., 2010). Therefore, the present method allows upward scalability in terms of one-pot green synthesis of fluorescent and biocompatible carbon dots, which can be used as fluorescent imaging probes for *in vitro* and *in vivo* bioimaging applications in cell biology and other sensing applications.

CHAPTER 6

CONCLUSION

Nanotechnology develops rapidly and assures innovations in many fields of science and technology. Over the last two decades, widespread research efforts have been conducted on carbon dots as one of the most extensively used classes of nanomaterials. Having their inherent mechanical, optical, biological, electrochemical and electrical properties, carbon dots have been extensively used in numerous areas. In addition, owing to their versatile surface properties, size and shape over the past decade, carbon dots have drawn great awareness in biomedical engineering. Hence, its interaction with biomolecules and response to the light will be simultaneously effective. The intriguing excellence of carbon dots is highlighted in their applications for bio-imaging including bacteria, stem cells and cancer cells.

In the present work, we described efficient and simple green synthetic techniques for the preparation of highly fluorescent carbon dots by using seaweed *Kappaphycus Alvarezii* extract as carbon source. The synthesized carbon dots exhibited an average size ~15 nm with intense bright blue color emission under UV-light, and these are characterized by UV-visible, photoluminescence, FT-IR, DLS and TEM techniques. The synthesized carbon dots conjugated with penicillin/streptomycin and showed high fluorescence ability for *in vitro* cellular imaging of *Escherichia coli* and *Staphylococcus aureus*. Therefore, the present method allows upward scalability in terms of one-pot green synthesis of fluorescent and biocompatible carbon dots, which can be used as fluorescent imaging probes for *in vitro* and *in vivo* bio-imaging applications in cell biology.

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