Evaluation of Biological Activities of Carbon Nanodots

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DECLARATION

I, Hafiza Noor Ul Ain, declare that all work presented in this thesis is the result of my own work. Where information has been derived from other sources, I confirm that this has been mentioned in the thesis. The work here in was carried out while I was a postgraduate student at Atta-ur-Rahman school of Applied Biosciences NUST under the supervision of Dr. Rumeza Hanif

Noor 03.08.2021

Hafiza Noor Ul Ain

Dedicated to My Sister

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CONTENTS

LIST OF ACRONYMS

3D	Three Dimensional
µm	Micrometer
nm	Nanometer
ml	Milliliter
mg	Milligram
rpm	Revolutions per Minute
kV	Kilo-Volt
MRI	Magnetic Resonance Imaging
CT	Computed Tomography
PET	Positron Emission Tomography
PCL	Polycaprolactone
QD	Quantum Dots
Fe^{+3}	Iron (III) Ion
RNA	Ribonucleic Acid
PVD	Physical Vapor Deposition
CVD	Chemical Vapor Deposition
LSPR	Localized Surface Plasma Resonance
UV	Ultraviolet
NLC	Nanostructure Lipid Carriers
SLN	Solid Lipid Nanoparticles
MOF	Metal-Organic Frameworks
CNT	Carbon Nanotubes
C_3N_4	Graphitic Carbon Nitride

MWCNTs	Multi-Walled Carbon Nanotubes
OH^-	Hydroxide Ion
LED	Light Emitting Diode
DOX	Doxorubicin
DNA	Deoxyribonucleic Acid
PEG	Polyethylene Glycol
CTC	Circulating Tumor Cells
FDA	Food and Drug Association
EpCAM	Epithelial Cell Adhesion Molecule
EMT	Epithelial-to-Mesenchymal Transition
RT-PCR	Reverse Transcription Polymerase Chain Reaction
BMSM	Brain Metastatic Selected Marker
HER2	Human Epidermal Growth Factor Receptor-2
AA	Ascorbic Acid
UV/Vis	Ultraviolet/Visible
SEM	Scanning Electron Microscopy
FTIR	Fourier Transformed Infrared Spectroscopy
KBr	Potassium Bromide
XRD	X-Ray Diffraction
MHA	Mueller-Hinton Agar
NA	Nutrient Agar
LB	Luria Broth
CND	Carbon Nanodots
CTX	Cefotaxime

CIP	Ciprofloxacin
DPPH	2,2-di-phenyl-2-picryl hydrazyl hydrate
µg/ml	Microgram per Milliliter
MCF-7	Michigan Cancer Foundation-7 (cell line)
MDA-MB-231	Metastatic Mammary Adenocarcinoma1 (cell line)
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
PBS	Phosphate Buffer Saline
EDTA	Ethylenediaminetetraacetic Acid
RPMI	Roswell Park Memorial Institute (culture media)
DMSO	Dimethyl Sulfoxide

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ABSTRACT

Carbon nanodots, due to their unique physicochemical properties have various applications in the field of biomedical sciences. Ranging from 1 to 20nm in size, the unique spherical quantum dots obtained from chemical and biological sources of carbon are significant for their striking fluorescence, a zero-dimensional morphology, homogeneity in size, the flexibility of surface passivation and for the promise of biocompatibility. These nanodots can be synthesized through a number of prescribed methods including the chemical methods of carbonization and the physical methods of breaking apart a carbon source using laser, heat or ultrasonic waves. This study has adopted the hydrothermal method with a Teflon-lined autoclave to synthesize CNDs by sealing ascorbic acid solution (carbon source) inside the autoclave and leaving it at 250°C for four hours. These nanodots were characterized with UV-Vis spectral analysis which gave a peak at 320nm and then another smaller one at 400nm. XRD displayed crystalline properties while SEM confirmed a spherical morphology and exceptionally small size of the nanodots. FTIR analysis showed the functional groups present at the surface of these nanodots which primarily included hydroxyl, aldehydic, ketonic and carboxylic groups. Antimicrobial activity against Escherichia coli, Staphylococcus aureus and Klebsiella pneumonia and antioxidant activity using DPPH assay were also determined to ascertain the possible applications for the synthesized CNDs. An almost non-existent cytotoxicity was determined through MTT assay to confirm that the synthesized CNDs were biocompatible. CNDs synthesized, characterized and evaluated here can be used for further applications in the field of biomedicine.

Chapter 1

INTRODUCTION

Nanoparticles are defined as distinct materials in the nanoscale hence constituting sizes between 0.1 to 100nm. Nanotechnology is the field of study of these very small particles owing to the interesting fact that their physicochemical properties at this size are very different from their properties at larger sizes (Simpson, 2015). This is primarily because the surface area to volume ratio is comparatively very high for nanoparticles as compared to materials at larger sizes. This uniqueness brings new and often previously unprecedented optical, physical and chemical properties into play.

Carbon nanodots (CNDs) are an integral part of the current advancements in nanotechnology owing to their fascinating properties which enable them to have a unique potential for great applications (Chen *et al*., 2019). Their surfaces can be adequately modified to include a number of different functional groups as per need or requirement of the study. Other than surface passivation, CNDs are also generally homogenous and have a zero-dimensional morphology which is also beneficial. CNDs also have compelling optical properties of fluorescence and bioluminescence which further enhance the range of their applications (Xu *et al.*, 2018). Moreover, the methods to synthesize CNDs are also varied and great in number which increases the chances of finding a method which suits numerous research facilities. However, the greatest advantage that comes with the synthesis and use of CNDs is that they are biocompatible and have very little to no toxicity in biological environments. This enables them to be uniquely possessed with the rather rare position in nanomedicine considering *in vivo* use (Sivasankarapillai *et al*., 2020). These advantages associated with CNDs have increased their popularity and simultaneously raised the amount of research performed on CNDs to properly understand their full potential and positively exploit their properties to the greatest extent.

There are wide range of synthesis methods for CNDs which range from chemical or conventional methods to more recently developed biological or ecofriendly methods. Over time, there has also been research and development in this field to lessen the number of steps needed to get to CND and to simultaneously increase effectiveness and applicable properties of the nanoparticles (Sivasankarapillai *et al*., 2020). Out of the various chemical methods for synthesis of CNDs, there is electrochemical synthesis which involves carbonization through

electrochemical means (Deng *et al*., 2014). Microwave method, also called the pyrolytic method, is another method for chemical synthesis of CNDs because the solution of the carbon source is just placed in the microwave and the heat carbonizes it (de Medeiros *et al*., 2019). Ultrasonic-assisted chemical methods are also used for the synthesis of CNDs as the ultrasonic waves carbonize the chemical source of carbon (Ma, *et al*., 2012). One of the most widely used methods is the hydrothermal method which includes the use of a hydrothermal autoclave which has the carbon source sealed in it in solution form and is placed at a certain temperature for a few hours which carbonizes the source (Zhang *et al*., 2018). This hydrothermal method of synthesizing CNDs is one of the most frequently used methods in this regard because of the ease of handling and the high quality of CNDs produced as a result; and also because other bottom-up methods for synthesis of CNDs require harsh conditions which makes them relatively undesirable (Baker and Baker, 2010). Simply placing a carbon source sealed in the autoclave at a certain temperature leads to carbonization in a controlled environment of temperature and pressure whereby the carbon source is converted into CNDs through a bottom-up procedure. All these methods for synthesis used for chemical sources including electrochemical method, microwave-assisted method, thermolysis, hydrothermal method and laser ablation method can also be applied to biological sources (Sivasankarapillai *et al*., 2020).

Characterization of CNDs involves a determination of the size, shape, physical and chemical properties of CNDs. The first of these is a confirmation of the fact that the synthesis process has been carried out successfully. One of the first indicators of the synthesis of CNDs is the faded yellowish-brown color of the solution which comes out of the hydrothermal autoclave (Sugiarti and Darmawan, 2015). This is in stark contrast with the colorless ascorbic acid solution which had been placed inside the autoclave at the start of the reaction. A burnt smell also accompanies this solution. The second part of initial confirmation is identification of the characteristic CND property of fluorescence. CNDs synthesized from a chemical carbon source in a bottom-up procedure like the hydrothermal synthesis give off bright blue fluorescence at UV light of wavelength 365nm (Qu et al., 2012). These CNDs have remarkable photostability so that this fluorescence persists even after continued exposure (Yang et al., 2013). Determination of the morphology and size of the CNDs synthesized is carried out using electron microscopy. Scanning electron microscopy (SEM) can be used for this. CNDs are understood to be spherical in size hence appearing circular in a SEM image with a diametrical size of about 10 to 20 nm (Sugiarti and Darmawan, 2015). UV/Vis

spectroscopy is carried out as part of determination of physical properties. CNDs are understood to have a similar peak to their precursor ascorbic acid but the critical difference is that the nanodots usually have an absorbance which is much higher between the wavelengths 300nm and 400nm (Sugiarti and Darmawan, 2015). The crystalline structure of the synthesized CNDs can be determined using X-ray Diffraction (XRD) analysis and the peaks can be seen at around 20 degrees (Gong *et al*., 2014). In order to gather more details about the surface chemistry of the synthesized CNDs, Fourier Transformed Infrared (FTIR) spectroscopy is carried out. Functional groups present at the surface are analyzed in this manner. Functional groups common on the surface of CNDs synthesized from ascorbic acid include the carboxylic acid groups and hydroxyl groups (Ortiega-Liebana *et al*., 2017).

CNDs have a number of biomedical applications owing to their physicochemical properties which make them perfect candidates for clinical and research-based studies in nanomedicine (Miao *et al*., 2015). One of these properties is photoluminescence which makes CNDs good candidates for biosensing. Heavy metal ions can be detected using CNDs which is significant considering the fact that these ions can accumulate in vital organs and lead to morbidities and complications. Chemiluminescence signals the presence of these metal ions (Zhao *et al*., 2014). Similarly, hydrogen peroxide is also dangerous in excessive quantities and its concentration can be figured out using quantification of the chemiluminescence by CNDs (Liu *et al*., 2014). Other biosensing applications include detecting the thrombin protein through aptamers attached to CNDs (Yoo *et al*., 2014). CNDs have an impeccable fluorescence property which makes then excellent candidates for bioimaging applications as well. Their fluorescence is stable over time and lengths of exposure, their water solubility is high, and their toxicity is low (Miao *et al*., 2015). In bioimaging applications, one example is detecting human serum proteins using gel electrophoresis (Na *et al*., 2013). In vivo detection and imaging of compounds like hydrogen sulfide can also be achieved using CNDs (Zhu *et al*., 2014). This is because the non-toxicity of CNDs and their small size and morphology leads to the situation where cells can internalize CNDs and the result gives off green fluorescence (Chandra *et al*., 2014).

However, the most significant biological property of CNDs which makes them perfect candidates for biomedical applications is the biocompatibility. This can be simply checked through the MTT assay or the cytotoxicity assay to observe whether CNDs have a significant cytotoxic impact on a human cell line (Gong *et al*., 2014). CNDs therefore become candidates for diagnostic applications. Development in the field of diagnostics is especially

groundbreaking in terms of cancer diagnostics and subsequent treatment. This is because detecting the presence of cancer at early stages is crucial in reducing the risk of fatality. Therefore, if nanomaterials, by virtue of their enhanced capabilities at the nanoscale, can increase the possibility of cancer diagnosis at an early stage, they are incredibly vital to the field of oncological research in general. CNDs are an example of nanoparticles that are making significant leaps in cancer research possible because of their physicochemical properties (Li *et al*., 2019). Out of the applications that CNDs have with respect to cancer, the contributions in diagnostics are considerable. CNDs can be conjugated with certain compounds or functional groups for bioimaging of cancer cells. An example includes conjugating CNDs with folic acid to detect cancer cells which were positive for folate receptors (Zhang *et al*., 2018). Some cancer cells overexpress glutathione which is a radical scavenger. CNDs conjugated with Fe^{-3} ions have been reported to be used for checking cancer cells which have high glutathione expression (Gao *et al*., 2018). Detection of glycoprotein as a biomarker of cancer through CNDs can also be carried out. Various CNDs surfaces have been functionalized to detect various glycoproteins which are markers for cancer. Glycosylated nanocarriers fashioned out of CNDs are one example (Das & Mohapatra, 2017). Detection of micro-RNAs as a cancer biomarker can also be carried out using CNDs by using a quencher so that the quenching can then be cancelled by the biomarker (Mohammadi & Salimi, 2018). Applications with respect to therapeutics linked with cancer include targeted drug delivery, photothermal therapy and photodynamic therapy (Li *et al*., 2019).

1.1 Hypothesis

Chemical synthesis of CNDs happens through carbonization of a carbon source at a certain temperature inside a sealed hydrothermal autoclave. Synthesized CNDs fluoresce and this property can be the defining characteristic for a successful synthesis. These nanodots are significantly small in size, are photostable and also have carboxylic and hydroxyl groups on their surface. Biological applications of CNDs are linked with their fluorescence and with the fact that they are remarkably biocompatible and nontoxic.

1.2 Objectives of the Study

Specific objectives of this research include:

- Chemical synthesis of CNDs from ascorbic acid using the hydrothermal method
- Confirmation of fluorescence property of newly synthesized CNDs
- Characterization of CNDs using UV-Vis, SEM, XRD and FTIR
- Optimization of antioxidant ability of CNDs using DPPH assay
- Observation of antimicrobial activity of CNDs against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*
- Confirmation of biocompatibility of CNDs using MTT assay for cytotoxicity

Chapter 2

Literature Review

2.1 Nanotechnology and Nanoparticles

Nanotechnology is a field of science which deals with extremely small particles, called nanoparticles, which traditionally range between 0.1nm to 100nm. Since the word "nano" means dwarf in Greek, its literal meaning associates with its scientific definition (Russell, 2013). Nanomaterials and nano devices can be used on the basis of their respective and distinct chemical reactivity, electrical conductance, optical effects, magnetism or physical strength (Nikalje, 2015). All these properties may be exploited in different arenas to yield beneficial results. This is because at extraordinarily small sizes, the physical properties of the same materials on macro-level change drastically as quantum effects dominate over classical physical properties (Idrees, 2015).

The history of nanotechnology can be traced back to 1959 when R. Feynman initiated the thought process behind nanotechnology (Vijayakumar *et al*., 2013). This was followed by Taniguchi using the term "nanotechnology" for the first time in 1974 (Taniguchi, 1974). After this, a series of inventions like the IBM Scanning Tunneling Microscope in 1981 and the bucky ball in 1985 furthered the scope of studying and searching for applications of extremely small materials. The National Nanotechnology Initiative came along in 2000 and soon, the Feynman award was being awarded for theoretical and applied innovations, discoveries and inventions in the field of nanotechnology (Petcu *et al*., 2007). 2005-2010 was the era of 3D nano systems. These included intersections with robotics or 3D networking. Active nano products were created which were capable of changing their physical or chemical state while being used. 2011 ushered in the era of molecular nanotechnology (Nikalje, 2015).

Nanoparticles include a very wide class of particulate substances which may have anywhere between zero and three dimensions (Tiwari *et al*., 2012). At least one of these dimensions should be less than 100nm (Laurent *et al*., 2010). Nanoparticles have distinct properties with respect to color, size and shape which can be varied with change in concentration and can be exploited for a range of applications including bioimaging (Dreaden *et al*., 2012). Since nanoparticles are very different from normal molecules, there are usually layers inside their structure. The outer layer is called the surface layer which can be functionalized by attaching

polymers, surfactants, metal ions or small molecules. These additions to the outermost layer give the nanoparticle unique functional properties which were nonexistent or diminished before. The second layer is usually called the shell layer which is chemically different from the other two layers. Innermost is the core which is the actual nanoparticle and responsible for its characteristics (Shin *et al*., 2016).

Synthesis of nanoparticles either goes top down which means that larger structures are broken down into smaller substances which lie in the nanoscale. Another approach is bottom up whereby individual molecules or atoms are arranged into nanostructures (Wang & Xia, 2004). The top down method is destructive whereby larger molecules are decomposed into smaller units and these units are then converted into nanoparticles. Top down methods include milling or grinding, physical vapor deposition, and chemical vapor deposition (Iravani, 2011). Bottom up involves synthesizing nanoparticles from simpler substances which means that it is building up from size smaller than that of nanoparticles. Examples of this approach include green synthesis, spinning, sol gel and biochemical synthesis which come under reduction and sedimentation techniques (Iravani, 2011). Laser irradiation can also be used as a bottom up approach because certain nanoparticles of a specific shape can be synthesized if reaction parameters like time for laser treatment are controlled within strict parameters (Liu *et al*., 2015). Solvent-exchange method is another example (Needham *et al*., 2016). Environmentally friendly synthesis methods which include biological systems as reducing agents also come under bottom up methods in nanotechnology.

Applications for nanoparticles can be found in a range of industrial, environmental and biomedical fields. Applications for nanoparticles in manufacturing and materials include marketable products in pharmaceutical and aerospace industries (Weiss *et al*., 2006). Health fitness products, food packaging and food processing industries are one of the most frequent users of nanoparticles for product manufacture. For printed electronics providing a feasible alternative to traditional silicon-based techniques, nanoparticles exist as a valuable starting material. Metallic nanoparticles, carbon nanotubes and ceramic nanoparticles are some examples of nanoparticles produced in bulk for electronic equipment (Kosmala *et al*., 2011). In the era of growing realization that the fossil fuel reserves are finite, nanoparticles are emerging as an important component in various processes for energy harvest including electrochemical or photoelectrochemical water splitting. Other examples of similar processes include electrochemical reduction of carbon dioxide for piezoelectric generators or solar cells (Ning *et al*., 2016). Nanoparticles can either be coated or embedded in metal or polymer

matrix which helps increase their mechanical strength (Kot *et al*., 2016). Mechanical properties of nanoparticles can be exploited for application in mechanical industries. They are also used for the production of sustainable products which are environmentally benign, remediation of contaminated materials and production of environmental sensors. Photodegradation using nanoparticles also falls in this category of environment-related applications of nanoparticles (Rogozea *et al*., 2017). Applications of nanoparticles exist in every branch of medicine. Drug delivery at optimum dosage, increased compliance in patient for the drug resulting in weakened side effects are achieved using nanoparticles (Alexis *et al*., 2008). Other than drug delivery, cancer diagnostics and therapeutics are other significant fields for use of various nanoparticles. Antibacterial efficacies of nanoparticles are also being exploited (Qu *et al*., 2016).

2.2 Physicochemical Properties of Nanoparticles

The fact that nanoparticles have such a wide array of applications in numerous fields is possible because of their diverse physicochemical properties enabling them to be useful in a variety of fields. Some of these are as follows:

2.2.1 Optical and Electronic Properties

The UV-visible absorbance and emission properties of nanoparticles are a notable indicator of the optical properties of said nanoparticles. Moreover, the electronic and optical properties of nanoparticles are linked and give rise to a unique property called localized surface plasma resonance (LSPR). LSPR results in a UV-visible excitation band that is unique to the nanoparticles and is not present in the spectrum of the metal from which the nanoparticles have been made. This band is formed because of an incident photon frequency which is constant with the way the conduction electrons show collective excitation. LSPR spectrum also has a distinct peak which is heavily dependent on the shape, size and the interparticle spacing in the nanoparticles. Dielectric properties of the nanoparticles and those of the environment including the solvent, adsorbates and the substrates are also important in determining LSPR spectrum peak (Eustis & El-Sayed, 2006). Gold and silver nanoparticles also express LSPR because the free electrons on their surface are freely transportable leading to no scattering in the bulk. This leads to standing resonance conditions when there is light interaction, hence causing LSPR.

2.2.2 Magnetic Properties

Research has confirmed that the magnetic properties of nanoparticles reach their optimum capacity at a size between 10 and 20nm (Reiss & Hütten, 2005). At this small size, these nanoparticles have magnetic properties strong enough to be used for biomedicine, water decontamination, MRI, magnetic fluids, catalysis (homogenous and heterogenous) and data storage. Magnetic properties of nanoparticles are understood to have risen from the uneven distribution of electrons in the nanoparticles. This property, however, depends on other factors as well. These include the protocol which has been used in the synthesis of these nanoparticles which can either be solvothermal, thermal decomposition, micro-emulsion, co-precipitation or flame spray synthesis (Wu *et al*., 2008).

2.2.3 Mechanical Properties

Distinct mechanical properties of nanoparticles are responsible for their use in surface engineering, tribology, nanomanufacturing and nanofabrication. The hardness, friction, adhesion, elastic modulus, stress and strain are parameters through which the mechanical properties of nanoparticles are determined. Mechanical properties of nanoparticles are enhanced or managed through lubrication, coagulation or surface coating (Guo *et al*., 2014). These properties are unique to nanoparticles and are not similar or close to the mechanical properties shown by microparticles or by the bulk material. There is also a very noticeable difference between the stiffness in nanoparticles and that of the bulk material when in a greased or lubricated contact. Surface quality can be enlightened, and material removal can be elevated if the mechanical features of nanoparticles are reasonably controlled. The parameters for determination of mechanical properties in nanoparticles are size dependent (Guo *et al*., 2014).

2.2.4 Thermal Properties

Metallic nanoparticles also have thermal properties that are distinct and unique when compared to those of the metals themselves. Fluids which have nanoparticles suspended in them have much higher thermal conductivity when compared to normal fluids carrying out conventional heat transfer. When solid particles in the nanometric scale are dispersed in solvents like water, oils or ethylene glycols, nanofluids are produced which are believed to have thermal properties much superior to those of fluids in their original form. Fluids having microscopic level particles also do not contain the thermal properties held by nanofluids. Nanoparticles with a larger surface area are more desirable for this purpose because the heat transfer in nanofluids happens at through the particle surface. Stability of the particle

suspension in the liquid is also enhanced if the surface area of concerned nanoparticles is large (Lee *et al*., 1999). Nanofluids made up of copper (II) oxide or aluminum (III) oxide nanoparticles in water as well as ethylene glycol have been shown to have remarkable thermal conductivity (Cao *et al*., 2002).

2.3 Classification of Nanoparticles

Nanoparticles can be classified into various groups based on their composition, functional properties, size, and morphology. Major groupings based on chemical composition are as follows:

2.3.1 Organic Nanoparticles

Organic nanoparticles primarily consist of lipid-based nanoparticles of various shapes and sizes (Zhong & Zhang, 2019). These include nanostructure lipid carriers or NLCs, solid lipid nanoparticles or SLNs, and liposomes. Since lipid-based nanoparticles have low toxicity and the ability to carry both hydrophobic as well as hydrophilic molecules, they are primarily used in drug delivery applications. Lipid-based nanoparticles can also be exploited to control drug release in specific targets inside the human body (Zhong & Zhang, 2019). Liposomes are made up of phospholipid compounds and cholesterol with a size range between 50 and 100nm. These nanoparticles have a high bioavailability and reduced toxicity which results in them becoming an ideal candidate for cytotoxic drug delivery operations. SLN molecules have been found to have limited capacity for the expulsion and loading of drugs. This is because SLN molecules tend to crystallize during storage. In order to counter this, NLCs with increased stability are preferred (Meikle *et al*., 2019). Hybrid nanoparticles called metal-organic frameworks (MOFs) consisting of organic ligands with inorganic metal ions are also used for beneficial properties like high porosity, high surface area, good organization of structure and configuration, and ease of surface modification (Gascón *et al*., 2018).

2.3.2 Inorganic Nanoparticles

Inorganic nanoparticles include metal nanoparticles, metal oxide nanoparticles as well as bimetallic nanoparticles. Nanoparticles of iron, gold and silver have been especially studied to have unique optical, chemical and electrical properties (Asghari *et al*., 2016). Properties of these metallic nanoparticles depend on the synthesis technique used. When particle sized is reduced until it is in the nanometer scale, its electronic structure changes because the bandgap

is shifted to certain electronic levels so that there is a large number of atoms on the surface of the nanoparticles. When the size of these nanoparticles is reduced, these extra atoms become active between unsaturated sites and the atomic coordinates. This activated surface characteristic of metallic nanoparticles is crucial for applications involving adsorption processes and catalysis. Catalytic activity of these metallic nanoparticles can be improved with the help of light irradiation (Kim & Lee, 2018).

Numerous metal oxides like SiO_2, ZnO, Al_2O_3, TiO_2 and Fe_2O_3 are routinely used to make metal oxide nanoparticles for applications as semiconductors, chemical sensors and catalysts (Saleh & Fadillah, 2019). These nanoparticles have distinct physical properties like a highly active surface area alongside their biocompatibility which makes them ideal for these applications. Surface modification of metal oxide nanoparticles can be done using organic compounds including thiols, amines, epoxies and anionic compounds (Hur *et al*., 2019). Bimetallic nanoparticles having two metallic components have unique properties with respect to reactivity and chemical stability hence making them suitable for a variety of applications in catalysis (Saha *et al*., 2019). The nanostructure of these nanoparticles is strongly influenced by whether the two metallic components are miscible.

2.3.3 Carbon-based Nanoparticles

Since carbon is a solid-state allotrope with numerous forms, the nanoparticles formed from carbon are significant precisely because of the wide range of shapes and structures in which they come. Graphite, amorphous carbon and diamond are all allotropic forms of solid carbon. Nanoparticles based on carbon can therefore have hybridized sp^2 atoms which can develop in a range of dimensions (Li *et al*., 2019). The chemical and physical properties shown by carbon-based nanoparticles include distinct chemical stability, mechanical properties, thermal properties, physical properties and conductivity etc. This diversity implies an equally diversified range of applications.

Carbon-based nanoparticles can be divided into numerous sub-categories based on the shape and based on the starting allotrope that they are based upon.

1. Fullerene nanoparticles are zero-dimensional nanoparticles having 60 carbon atoms arranged in the form of a buckyball and having properties capable of neutralizing species like oxygen and nitrogen (Sumi & Chitra, 2019).
2. Another significant type is carbon nanotubes or CNTs which are one-dimensional material which has a hollow structure made up of regularly arranged carbon atoms in

the form of hexagons. CNTs are used for various applications including enhancement of thermal stability and mechanical strength owing to the unique physical properties of these nanotubes. Chemical vapor deposition is the synthesis method for CNTs during which, the homogeneity and size of the CNTs can be controlled (Battaglia *et al*., 2020).

3. Graphene sheets are another example of two-dimensional carbon-based nanoparticles where the carbon structures create a honeycomb lattice through hexagonal arrangement. The high electrical conductivity, large surface area, chemical reactivity and good stability of graphene sheets are properties contributing to unique applications (Vesel *et al.*, 2019).
4. Nano-diamonds having exceptional mechanical stability with a sphere-like layered shape have distinct magnetic and optic properties making them suitable for use as abrasives, semiconductors and in coating (Yan *et al*., 2016).
5. CNDs are another type of carbon nanoparticles notorious for their unique properties and applications.

2.4 Carbon Nanodots (CNDs)

CNDs are significant in the realm of carbon nanoscience owing to their extraordinarily small size (~10nm) and a tunable, bright fluorescence. These nanoparticles were discovered in the 2000s and soon gained importance because of their unique properties and potential for exploitation (Sun *et al*., 2006). The ability of CNDs to fluoresce is important because it permanently shifted the traditionally held position of understanding carbon as a black solid which is incapable of emitting light. Non-toxicity, biocompatibility, high water solubility, low cost, ease of synthesis, electron donor or acceptor qualities and the capability of being highly sensitive to external environment has earned CNDs their value in nanotechnology. It is this combination of favorable properties which makes CNDs significant with respect to a range of applications ranging from imaging and sensing to optoelectronics. Optical properties of CNDs are comparable in application to fluorescent quantum dots in the sense that CNDs are much better in terms of resistance to photobleaching, biocompatibility, low toxicity and aqueous solubility (Cayuela *et al*., 2016). Moreover, as procedures for synthesizing CNDs are progressively getting better and being optimized, their quantum yields are also increasing. Good absorption in the blue and UV range without any serious blinking drawbacks are defining characteristics of CNDs (Li *et al*., 2012).

When describing the structural configuration of CNDs, it is quite evident that CNDs actually have numerous structural subcategories which are all different variations of a quasi-spherical morphology. Their structure can be graphitic, it can have a C_3N_4 crystalline core, and it can be amorphous (Cayuela *et al*., 2015). Sometimes, graphene quantum dots consisting of nanoscale fragments of graphene sheets are also considered as a subtype of CNDs. This is significant because even though CNDs are zero-dimensional and graphene quantum dots are two-dimensional, their photo-physics are similar to those of CNDs (Shinde & Pillai, 2012). All CNDs, no matter their synthesis procedure, have a passivated outer layer which is, in some instances, a few nanometers thick. This passivation layer, having a layer of external agents all arranged around and bound to the carbonaceous core, is an integral part of the functionality and the structure of the CNDs (Bourlinos *et al*., 2008). Surface chemistry and specific structure of respective CNDs can confirm whether they are hydrophilic or hydrophobic in nature. Amid this diversification within CNDs, the constant properties present in all CNDs are their uniquely small size (~10nm) and the functionalization layer on their surface which tends to be highly disordered and dense (Sciortino *et al*., 2017). Both of these properties are crucial in producing the defining photoluminescence of CNDs because even though the functional units on the surface are not fluorescent themselves, they are extremely important in causing and regulating the fluorescence emitted by CNDs.

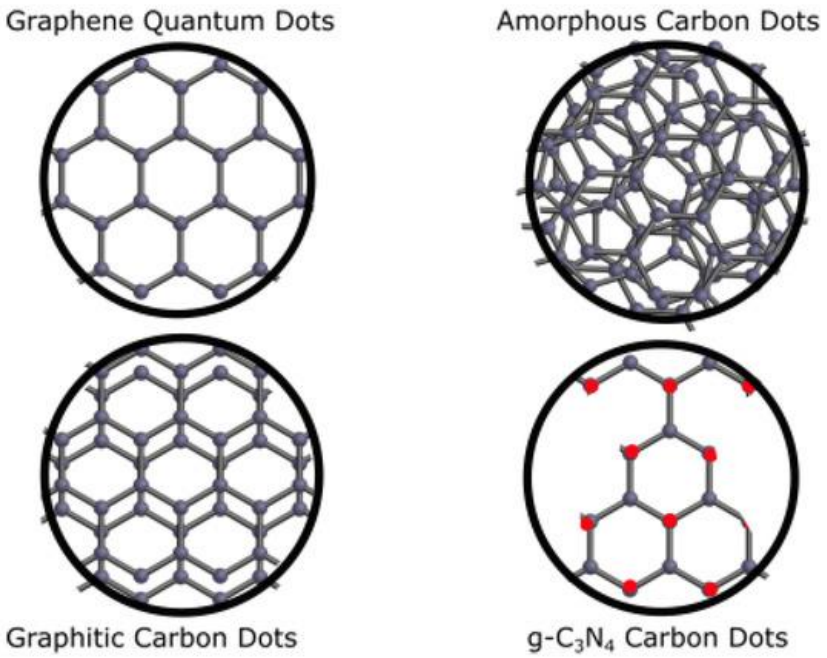

Figure 2.1: Commonly reported structures of CNDs (Sciortino *et al*., 2018).

Optical properties of CNDs are also variable as CNDs synthesized using different procedures may have the fluorescence at different wavelengths. There are types of CNDs capable of emitting blue, green and red light respectively which depends on the synthesis procedure as well as the excitation wavelength. This fluorescence is tunable because the emission peak continues to shift when excitation wavelength is changed. (Liu *et al*., 2012). Moreover, the intensity of their fluorescence is also not constant as it is subject to change under influence of ions in the solution. Interaction with other nano-systems may also lead to a decrease in fluorescence intensity. Sometimes, fluorescence of CNDs is completely lost with the production of nanodot aggregates in the solution (Zhou *et al*., 2017). CNDs have fluorescence which highly depends on the peak as well as the shape of the emission band and also on the solvent polarity (Sciortino *et al*., 2017). If metal ions are present in the solution holding CNDs, it also affects their fluorescence which can either be quenched or enhanced based on the specific structure of the nanodot and the identity of the metal ion. Other molecules in the solution may also impact this fluorescence.

2.5 Synthesis of CNDs

There are numerous methods which are routinely used to synthesize CNDs. All these methods fall in either one of two categories: top-down approach where larger molecules are broken down, and bottom-up approach whereby smaller atoms come together to make CNDs. Both methods have their own advantages and disadvantages. Top-down methods are easier to control and the CNDs produced this way usually have well-defined structures which are simple and there is no structural ambiguity in there (LeCroy *et al*., 2014). Bottom-up methods, on the other hand, are more difficult to control in terms of the structure and functionality of resultant products because side products produced as a result may quench the fluorescence. Intense purification steps are needed to prevent this. The advantage, however, is that this type of synthesis is simpler and easier with surface passivation often happening alongside the synthesis (Sciortino *et al*., 2018).

Figure 2.1: Synthesis Methods to Prepare CNDs (De & Karak, 2017).

2.5.1 Laser Ablation

This is a top-down method of synthesis of CNDs in which a pulsed laser beam interacts with the target surface of the solid precursor resulting in ejection of nanoparticles (Amendola & Meneghetti, 2013). When a graphite target is ablated with a laser beam under specific conditions, it produces carbon nanoparticles in the form of aggregates. These nanoparticles are not fluorescent and gain photoluminescence only after these nanoparticles have been treated with acid and then have had their surface passivated with organic molecules. Once this is done, the nanoparticles become CNDs. Characteristics of these CNDs can be modified and controlled if the conditions of synthesis are changed. Variations in the pulse duration changes the morphology of core structures and hence changes the size of the resultant CNDs. This size variation is achieved because when pulse duration is changed, the process of nucleation and growth changes (Hu *et al*., 2011). The wavelength of the laser beam used for ablation also has an impact on the size and fluorescence of resultant CNDs. The quantum yield of larger CNDs with crystalline grains starts decreasing with increase in size.

2.5.2 Electrochemical Synthesis

This is also a top-down method which involves use of an electrochemical cell in which a redox reaction is taking place under the influence of a current with a liquid electrolyte and two solid electrodes (Therese & Kamath, 2000). Multi-walled carbon nanotubes or MWCNTs have been shown as a portable electrode in this process. If the precursors used here are graphitic in nature, the resulting CNDs will also be prevalently graphitic in nature. The

impact of pH on this reaction was discovered when it was shown that an alkaline electrolyte with inert graphite electrodes successfully produced fluorescent CNDs while the same setup in acidic conditions failed to do so (Li *et al*., 2010). This was explained with the idea that the OH^- groups in alkaline solution cause the oxidation and formation of CND surfaces which are, in turn, crucial for the luminescent properties. Changing the time of the electrochemical synthesis can also change the size, morphology and fluorescence of the CNDs. Moreover, if the voltage applied is increased, CNDs of smaller size are produced (Bao *et al*., 2011).

2.5.3 Microwave Pyrolysis

Microwave pyrolysis is a bottom-up approach which has good commercialization and produces good quality CNDs with an easy, fast and simple procedure (Shen *et al*., 2018). A simple carbon source can be used alongside an organic polymer for surface passivation to synthesize CNDs that already possess a functionalized surface. The photoluminescence properties of the CNDs produced in this manner is dependent on excitation wavelength. This method is also environmentally friendly which increases its desirability. The CNDs produced in this way have numerous oxygen-containing groups on their surface that become sites for further attachment of metal ions. This interaction is important for the production of electrocatalysts (Wang *et al*., 2019).

2.5.4 Hydrothermal/Solvothermal Synthesis

Hydrothermal method is another bottom-up approach for the synthesis of CNDs and is the most frequently used and reliable method for this purpose (Liu *et al*., 2018). When CNDs are synthesized using a hydrothermal autoclave, the setup is straightforward, and the product formed in this manner is usually of uniform size with a significant quantum yield. The reaction precursor is usually a small organic compound, or a polymer dissolved either in an organic solvent or water. This reaction mixture is placed inside a Teflon-lined hydrothermal autoclave made of stainless steel. The setup is placed at high temperature for a certain amount of time. It results in the organic molecules coming together to first form the carbon seeding cores and slowly grow into functional CNDs of size around 10nm (Anwar *et al*., 2019). Nitrogen doping during synthesis is achieved by putting a nitrogen source alongside a carbon source in the reaction mixture. This results in dramatically higher quantum yields. It is apparently because of the fact that it adds another electron trapping surface to the nanodots. This results in higher radiative recombination and quantum yield rises. Nitrogen doping, when done on higher levels, sometimes also leads to the creation of carbon nitride

nanocrystals which are unique to bottom-up hydrothermal method (Bhattacharyya *et al*., 2017).

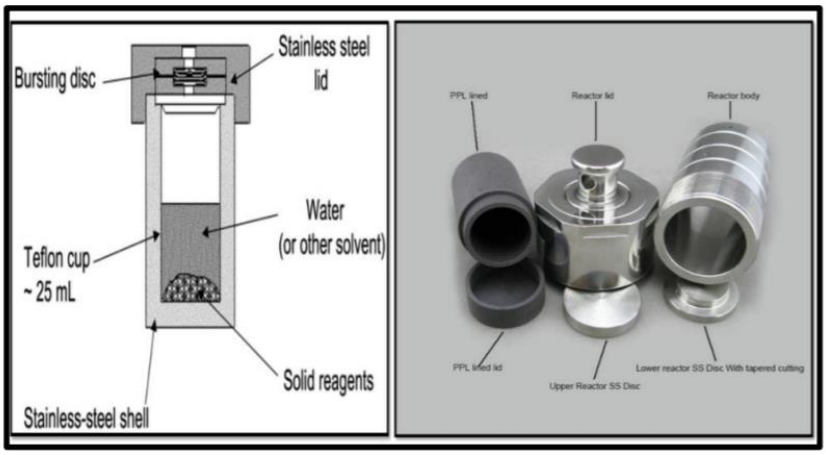

Figure 2.2: Schematic illustration of a Teflon-lined stainless steel autoclave (Einarsrud & Grande, 2014).

2.6 Applications of CNDs

CNDs can have a host of applications due to their physicochemical properties. These nanoparticles have both the qualities of optically active quantum dots while having the biocompatibility and non-toxicity of carbon-based materials at the same time. Applications of CNDs therefore cover a surprisingly wide range.

2.6.1 Nano-sensing

CNDs are being fast recognized as impressive components of nano-sensors because of the unique ways in which their sensitivity towards their environment can have an impact on their photoluminescence. These nano-sensors can either be comprised purely of CNDs in their original synthesized and passivated form. Another possibility for these nano-sensors is to have a fabricated nanocomposite created by coupling CNDs with micro or nanomaterials. There are heaps of literature on use of CNDs as raw sensors for ions, organic molecules and macromolecules in both chemical and biological fields (da Silva & Gonçalves, 2011). This is usually achieved by quantifying the impact of these ions or molecules on the fluorescence capability of CNDs. The unique fluorescence quenching caused by chemical changes in the environment around CNDs is the main principle behind use of these nanoparticles as sensors. CNDs are usually very good sensors because they exhibit high sensitivity towards changes in their environment and hence even a small concentration of detectable ions produces

discernable results (Liu *et al*., 2013). Selectivity in these experiments is also high. There is a dearth of proper understanding with regards to the reason behind this quenching, so the experiments usually proceed on a hit-and-trial basis. However, a preliminary understanding insists that it is because of electronic transfer from the nanodots to the ions resulting in a hindrance to the radiative recombination responsible for fluorescence (Liu *et al*., 2018). Literature usually contains the use of CNDs for detection of metal ions and it is considerably rarer to find such work on sensing anions which is because CNDs are already negatively charged and the presence of anions works in reverse as it removes the quenching effect of metal ions on CNDs (Hu *et al*., 2015). CNDs are understood as ratio-metric sensors because of dual emission.

2.6.2 Optoelectronic Devices

CNDs have strong adsorption qualities, are capable of donating and accepting electrons, and have tunable emission. This leads to their potential use in optoelectronic devices promising. CNDs are either used in the form of fluorescent downconverters or in electroluminescent devices as the active layer (Ding *et al*., 2017). The first of these two is made possible by the fact that CNDs absorb light in the UV and blue range emitting it in the visible range with this emission being tunable enough for use in LEDs (light emitting diodes). The second application depends on the property of electroluminescence in CNDs. CNDs are also being used in the production of solar cells because of a long tail of visible absorption present in these nanoparticles due to which, solar light can be easily harvested using them. The capability of easy electron acceptance and donation in CNDs makes them excellent candidates for the position of sensitizer in solar cells (Mihalache *et al*., 2015). Similarly, if a CNDs intermediate layer is added to a solar cell, electron transport is sped up.

2.6.3 Photocatalysis

Photocatalysts speed up chemical reactions when they are excited in the presence of light. CNDs are active candidates for applications in photocatalysis because of adsorption qualities, the ability to be coupled with other materials including metal oxides as well as the ability to easily donate and accept electrons (Hutton *et al*., 2017). CNDs are used as photosensitizers which means that when CNDs absorb light, they transfer electrons to semiconductors. These semiconductors would have only been capable of absorbing the electron in UV. The presence of CNDs makes it possible for the catalysis to happen in visible light. Another use for CNDs in this field is when they accept charge carriers coming from the semiconductor which is

already photoexcited. This leads to suppression to a recombination of the electrons and holes hence increasing the efficiency of the catalysis (Xie *et al*., 2014). Other than this use of composites made up of CNDs and semiconductors, CNDs alone can also be used as photocatalysts in reactions like carbon dioxide conversion or water splitting (Cao *et al*., 2011).

2.6.4 Biological and Nanomedical Applications

Nanomedicine is an interdimensional field which brings together the innovation of nanotechnology to the field of biomedicine (Bayford *et al*., 2017) This application of nanotechnology has spectacularly expanded the potential of biomedicine because there are seemingly endless possibilities and directions in which nanomaterials can be put to use. Currently, nanoparticles of various chemical compositions and morphological structures are being used for both diagnostic and therapeutic purposes. In the field of diagnostics, nanotechnology has made great advancements in Magnetic Resonance Imaging (MRI), Computed Tomography (CT), Positron Emission Tomography (PET) and in the field of bioimaging in general. When it comes to therapeutics, there are antimicrobial nanoparticles, there are nanoparticles in development of therapies for diabetes and most importantly, there are nanoparticles in development of therapies for cancer. A huge part of this therapeutics subcategory is the ability of nanoparticles to be used as a drug delivery system. This includes nanoparticles that are polymeric in nature, liposomes, micelles as well as hybrid nanoparticles. Nanotechnology is also being put to use in regenerative medicine which especially includes nano-hydroxyapatite and AuNP-blended polycaprolactone (PCL) (Saxena *et al*., 2020). Nanomedicine is also becoming a considerable player in the pharmaceutical end of the biomedical industry (Rizzo *et al*., 2013). It is therefore clear that nanomedicine encompasses a wide variety of applications which may help researchers and clinical professionals to examine, control, repair and enhance the natural frameworks in the human body. This primarily means that the safeguard and enhancement of human well-being is being ensured through advancements in the field of nanomedicine. However, the same unexpected physical and chemical properties which bring unforeseen advantages to the world of biomedicine can also become likely liabilities to biomedicine specifically, and to the global ecosystem in general. With respect to unexpected consequences in the realm of biomedicine, nanoparticles could also have unhealthy side effects or causative impact on certain morbidities which were not necessarily expected beforehand (Nikolova *et al*., 2020).

Therefore, any research and development in the field of nanomedicine is to be undertaken considering the precarious balance between risk and reward.

Traditional quantum dots have been used for biomedical applications but CNDs are newer and much better alternatives. This is because CNDs have fluorescence qualities while also being non-toxic when the greatest problem with other quantum dots was their toxicity.

2.6.4.1 Drug Delivery

Drug delivery systems are usually designed based on the idea that drugs are to be delivered to certain part of the body and that there should be feasible interaction between the drug and the target. When nano-systems are conjugated with drugs, there is an overall improvement in drug delivery because there is better absorption, distribution followed by better elimination (Khan *et al*., 2015). CND are very small in size resulting in considerable cell membrane permeability which, when combined with their fluorescence, makes them worthy candidates for drug delivery systems. Hollow carbon quantum dots are also important here because drugs like doxorubicin (DOX) can be loaded into the hollow carbon dots and a pH-controlled release confirms that it is an appropriate drug delivery system. CNDs can also become magnetic when they are part of a drug delivery system hence becoming important in magnetic resonance imaging (MRI) (Yao *et al*., 2017). Fluorescence of CNDs makes MRI results better. Simultaneous co-delivery of more than one drug is also possible with CNDs (Zhang *et al*., 2017).

2.6.4.2 Gene Delivery

Appropriate gene vectors are a crucial component of all gene therapy ventures because correction of the root cause of genetic defects is not possible if the gene is not correctly delivered for the effect to actually happen. Numerous nanoparticles including quantum dots have been used as gene delivery vehicles (Ghafary *et al*., 2017). Because of biocompatibility, broad excitation spectra, strong fluorescence emission, stable photoluminescence, and low toxicity make CNDs suitable candidates for this same purpose. There is a capacity for condensing plasmid DNA in CNDs with remarkable efficiency in the transfection process. Clathrin or caveolae-mediated pathways for endocytosis can be used for cellular uptake of the complex made up of plasmid DNA and CNDs (Zhou *et al*., 2016).

2.6.4.3 Bioimaging

Nanoparticles are routinely conjugated with organic molecules like antibodies, peptides or small molecules in order to detect cancer cells or to act as biomarkers (Kairdolf *et al*., 2013). Quantum dots having narrow emission and broad absorption spectra with a high enough quantum yield are also used as fluorophores for bioimaging. CNDs are superior because of physicochemical stability, non-blinking characteristics, water solubility, non-toxicity and biocompatibility. This is why CNDs are routinely used for *in vitro* imaging (Lei *et al*., 2016). The incredibly small size of CNDs makes it possible for them to probe biological structures too small for other nanoparticles and this lowers the volume of *in vivo* injections needed to get it done. When CNDs are incubated with human cancer cells and excited at 405nm, luminescence from inside the cells can be seen which shows excellent cell permeability for CNDs. Photoluminescence of CNDs does not diminish during the excitation time needed which confirms that the resistance towards photobleaching means higher photostability which further makes *in vitro* bioimaging through CNDs better (Hu *et al*., 2014). CNDs synthesized even from toxic precursors are not toxic themselves while the use of natural sources or food products for synthesis of CNDs lowers their toxicity and makes them more desirable for biomedical applications (Molaei, 2019). CNDs are also used for *in vivo* imaging during which, longer wavelengths are preferred because it increases photon-tissue penetration while decreasing background autofluorescence. Excitation at wavelengths equal to or higher than 595nm results in improvement in separation between background and signal (Tao *et al*., 2012). CNDs have higher absorptivity as compared to conventional quantum dots and this is especially true for CNDs which have been passivated with PEG. This way, even though fluorescence yield for CNDs is lower at longer wavelengths, the upsides are enough to make up for it (Molaei, 2019). Photoacoustic imaging which integrates optical excitation of CNDs with ultrasonic detection is done *in vivo* with a single probe which can lead to better tissue penetration when increasing imaging sensitivity. During long circulation time, CNDs accumulate in tumors (Ge *et al*., 2015).

Chapter 3

Materials and Methods

The research is based on the chemical synthesis, characterization and applications of CNDs. The research was carried out in the Cancer Biology Lab of Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Science and Technology (NUST), Islamabad. The materials and methods used in this research are as follows:

3.1 Synthesis of CNDs

The synthesis of CNDs was carried out using the method of hydrothermal carbonization of ascorbic acid. This procedure has been reported in literature for a yield of highly fluorescent CNDs (Ortega- Liebana *et al*., 2017). Their method was slightly modified in the purification steps.

3.1.1 Hydrothermal Carbonization

In order to carry out the procedure of hydrothermal carbonization, a Teflon lined stainless steel autoclave (TI0100) was used. The hydrothermal reactor used here is a metal autoclave having Teflon lining and may sometimes also contain a gold, platinum or silver tube which shields the body of the autoclave from the corrosive compounds which may be in the reactant mixture in it.

3.1.2 Synthesis Reaction

Since AA was the carbon source in this process, 1M solution was prepared by adding 1.761g of Ascorbic Acid (Sigma Aldrich, Germany) in 10 ml of deionized water. This mixture was placed on the magnetic stirrer for 5-10 minutes at 50-60°C so that it could be properly dissolved. The resulting solution was poured in the Teflon lined autoclave which was sealed fully in order to avoid leakage. This autoclave was then placed in an oven incubator at 250°C for four hours. Once this reaction was completed, the autoclave was cooled back to room temperature and the solution inside was subsequently retrieved. This solution was supposed to be yellowish brown in color with a burnt scent. The solution was centrifuged at 6000 rpm for 90 minutes and the agglomerates formed during the carbonization process were therefore removed. Further purification of the supernatant from this centrifugation was carried out using a 0.22 µm syringe filter. This filter removed large particles which were unable to pass

through the filter. The solution obtained after this step was dark yellow/light brown in color. This solution contained AA CNDs.

3.2 Confirmation of Fluorescence

In order to confirm the fluorescence property of CNDs, the centrifuged and filtered solution was diluted to 4 mg/ml and 2 mg/ml concentrations. These solutions were put in cuvettes while a reference solution having an ascorbic solution in a cuvette was used. These dilutions were then put under UV of wavelength 365 nm using a UV DOC (Wealtec Dolphin DOC) and their pictures were taken.

3.3 Optical Properties of CNDs (UV-Vis Spectroscopy)

The optical properties of CNDs were measured using UV-Vis absorbance spectroscopy which means that absorbance peak obtained from the sample was analyzed to confirm the synthesis of CNDs.

3.3.1 Sample Preparation

In order to obtain the absorbance peak for the CNDs, a cuvette was filled up to its three quarters with the CNDs solution. This cuvette was then placed in the UV-Vis spectrophotometer (model AE-S90-2D from A & E Lab., UK). The blank or reference solution in this process had a similar volume of deionized water. The nanodots absorbed photons of certain wavelengths and the machine plotted their absorption spectra. The wavelength range for this process was set at 200-800 nm and the graph obtained on the computer attached with the machine was observed to confirm that the solution contained CNDs.

3.3.2 Concentration Measurements

Concentration determination is essential for ascertaining the biological applications for CNDs. Since calculating the accurate concentration of CNDs is so important, it was measured using the Beer-lambert law. According to this, $A = \varepsilon \times C \times 1$. For this formula to work, A is the absorbance for a certain wavelength, C is the concentration of the CNDs in moles per liter, 1 is the path length and ε is the moral extinction co-efficient of CNDs. This value is 1.0×10^5 $M^{-1}cm^{-1}$ for carbon atoms that are π-conjugated (Kelarakis, 2014). The formula was therefore used to find out the concentration of CNDs synthesized.

3.4 Characterization of CNDs

The synthesized CNDs were characterized so that their physical and chemical properties could be determined. An insight into their physical and chemical properties confirmed that the CNDs manufactured in this study aligned with the properties which have already been reported in literature.

3.4.1 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was performed on CNDs to check their morphology and size. For this purpose, the machine company and model were Jeol JSM-6490A. The resulting images from this machine were supposed to give an insight into whether CNDs of the correct size had been reported.

3.4.1.1 Sample Preparation

In order to prepare the sample for SEM, the prepared CNDs solution was used. One drop of this solution was added in 10ml deionized water and the solution was poured in a glass vial. This glass vial was then placed in an ultrasonicator (Cole-Parmer) for 1 hour. This step was carried out so that the agglomerates in the solution are broken and the nanodots are properly suspended in the solution in an even distribution. One drop of this sonicated sample was then placed using a micropipette on a small 1 cm × 1 cm glass slide. This drop was then dried by placing it directly under a lamp until the water had evaporated. The dried and fixed sample was then coated with gold so that the surface was conductive. This was done using ion sputtering device and auto coater (Jeol JFC-1500, Japan). Once the samples were coated, conductive tape was used to place them on stubs. Scanning took place with voltage at 10kV and magnification at 50,000.

3.4.2 Fourier Transform Infrared Spectroscopy (FTIR)

The functional groups present at the surface of synthesized CNDs were determined using Fourier Transform Infrared Spectroscopy (FTIR). This was done using the Perkin-Elmer Spectrum-100 spectrometer.

3.4.2.1 Sample Preparation

The sample for FTIR analysis of synthesized CNDs was prepared using KBr pellets. For this purpose, the solution containing synthesized CNDs was centrifuged to obtain a pellet which was later dried. This pellet was mixed with KBr and a pellet was prepared using a hydraulic

press. The pellet thus obtained was placed in the spectrometer. In order to analyze the functional groups present on the surface of the CNDs, the Perkin-Elmer Spectrum-100 spectrometer was set to scan at a wavelength range from 450 to 4000 cm^{-1}.

3.4.3 X-ray Diffraction Spectroscopy (XRD)

This technique was used to identify and analyze the presence of crystallinity in the synthesized CNDs. In order to do this, a D8 Advance DAVINCI design from Bruker, Germany was used to analyze the carbon nanodot sample.

3.4.3.1 Sample Preparation

In order to prepare a sample for X-ray Diffraction (XRD) Spectroscopy, a drop of the synthesized CNDs solution was placed on a glass slide. This drop was then placed in the oven for 10-15 minutes to dry it. The drop was then concentrated by placing another drop on top of it and drying that as well. Once the drop was sufficiently concentrated, the sample was taken to the machine and placed in the XRD chamber. The data obtained from the machine was analyzed using the Origin software in order to get a plot.

3.5 Antibacterial Activity of CNDs

Antibacterial activity of nanoparticles is related to their therapeutic capabilities and can be later incorporated into the relevant biomedical application as per requirement of a certain situation. Even though the primary biomedical application often associated with carbon dots is biosensing and bio-labeling, the antibacterial activity of carbon dots has also been extensively reported in literature and can be a helpful addition to the various other functions that carbon dots perform (Jhonsi *et al.*, 2018). This antibacterial activity was evaluated in this study using the well diffusion assay. Since the well diffusion assay is a procedure meant to quantify the ability to inhibit bacterial growth, it is appropriate for measuring the antimicrobial activity of carbon dots. Here, the protocol was used on three bacterial strains including *Staphylococcus Aureus* (gram-positive), *Escherichia Coli* (gram-negative) and *Klebsiella pneumonia* (gram-negative).

3.5.1 Preparation of the Mueller-Hinton Agar (MHA) Media

In order to prepare the Mueller-Hinton Agar (MHA) media, 38g of Mueller-Hinton agar (HiMedia Laboratories, India) was added to 1000 ml distilled water. This was followed by autoclaving the media to sterilize it at 121°C. After sterilization, it was cooled down to

around 40-50°C and poured in previously autoclaved petri dishes inside laminar flow hood so that contamination could be avoided. Once the media in the petri dishes was solidified again, the dishes were sealed and incubated at 37°C. This kept the plates sterile for the experiment.

Ingredients	**Concentration (g/L)**
Beef Extract	2
Starch	1.5
Acid Hydrolysate Casein	17
Agar	17

Table 3.1 Composition of MHA media

3.5.2 Preparation of Nutrient Agar (NA) Media

In order to prepare the Nutrient Agar (NA) media, 28g of nutrient agar (HiMedia Laboratories, India) was dissolved in 1000 ml distilled water. This media was then sterilized by autoclaving at 121°C and poured into petri plates which had also been sterilized by a similar method. This hot, liquefied media was allowed to be solidified again so that bacterial isolates could be grown on them.

Ingredients	**Concentration (g/L)**
Yeast Extract	1.5
Beef Extract	1.5
Agar	15
Peptone	5
Sodium Chloride (NaCl)	5

Table 3.2 Composition of Nutrient Agar Media

3.5.3 Preparation of Luria Broth (LB) Media

The Luria Broth (LB) Media was prepared so that the bacterial isolates could be grown in falcon tubes when the media gets turbid which is an evidence for bacterial growth. LB media was prepared when 10g of tryptone (Merck, Ponda, Goa), 10 g of Sodium Chloride or NaCl (Sigma Aldrich, USA) and 5g of yeast extract (Merck, Ponda, Goa) were all added to 1000ml distilled water. Before autoclaving this mixture at 121°C, it was heated, and the constituents were mixed properly. After sterilization, the media was poured in 15ml falcon tubes where the bacterial isolates were to be grown.

Ingredients	Concentration (g/L)
Tryptone	10
Sodium Chloride (NaCl)	10
Yeast Extract	5

Table 3.3 Composition of Luria Broth Media

3.5.4 Collection of Bacterial Isolates

Three bacterial isolates were collected including *Escherichia coli* (gram-negative), *Klebsiella pneumonia* (gram-negative) and *Staphylococcus Aureus* (gram-positive). These isolates had been provided by Microbiology Lab, ASAB, NUST, Islamabad. Before performing the well diffusion assay on them, all three of these isolates were grown on petri plates.

3.5.5 Inoculation of Bacterial Isolates

The three bacterial isolates were individually taken from pure cultures using wire loops and were used to inoculate the LB broth which had been prepared and stored in 15ml falcon tubes before this. Once inoculated, the falcon tubes were left for the bacteria to grow at 37°C. Once it was turbid, it was clear that the bacteria had grown in the media.

3.5.6 Preparation of CNDs Concentrations

In order to prepare a concentration of CNDs (CNDs) appropriate for the well diffusion assay, a pellet of CNDs was dispersed in 1 ml of deionized water with the help of a high-speed vortex. After the vortex, the solution was sonicated for 1 hour. Several concentrations of CNDs were made in different Eppendorf tubes which were 35 µg/ml, 45 µg/ml and 55 µg/ml. The three dilutions would test the antimicrobial activity of CNDs.

3.5.7 Well Diffusion Assay

This assay was performed in the laminar hood to analyze the antimicrobial activity of CNDs in a sterile environment. In order to further avoid any contamination, UV light was left turned on in the laminar hood for fifteen minutes before working in it. For this assay, the petri plates which had solidified Mueller-Hinton Agar (MHA) media were used. Each plate was marked into four sections including 3 sections for the 35 µg/ml, 45 µg/ml and 55 µg/ml concentrations of CNDs while the fourth section was for negative control. This negative control was deionized water. The same division into sections and use of negative control was done for all three bacterial strains. Other than these three plates, another fourth plate was used

for positive control and had two sections. One of these sections had the antibiotic Cefotaxime (CTX) (Oxoid Ltd., UK) while the other had the antibiotic Ciprofloxacin (CIP) (Oxoid Ltd., UK). These four labeled petri dishes were then cultured using cotton swabs which had been sterilized and then dipped in LB broth which had the bacterial isolates. These cotton swabs were spread over the surface of the MHA media so that the whole surface had the bacterial culture on it. Using a sterile tip, a well having a diameter of around 6-8 mm was punched in the solidified media after which the dilutions of 35 µg/ml, 45 µg/ml and 55 µg/ml were poured in these wells. In the section labeled negative control, deionized water was poured instead of the CNDs. For the plate with the positive control, there were two sections and therefore two wells. One of these wells was given CTX antibiotic and the other had CIP antibiotic. All of these plates were incubated at 37°C for 24 hours. After this had been done, zone of inhibitions which appeared on these plates were analyzed. The experiment was performed in triplicates (Balouiri *et al*., 2016).

3.6 Antioxidant Activity of CNDs

Antioxidant property of the CNDs was measured to check their ability to scavenge free radical (Zhang *et al*., 2017). In order to do it, the DPPH (2,2-di-phenyl-2-picryl hydrazyl hydrate) assay was used. DPPH is a calorimetric as well as quantitative substance which, when oxidized, changes color from purple to yellow. This color change happens because of the formation of picrylhydrazine molecules which are yellow in color. The reduction that causes this color change is also observable because of spectral analysis using the UV-Vis spectrophotometer at 517nm wavelength. The quantification in this assay is dependent on the inhibition percentage of free radicals which have been formed by the antioxidant. The principle behind this assay is that an antioxidant such as AA is a hydrogen donor so when it is present in a solution and the DPPH reagent is added to it, the DPPH interacts with the antioxidant and reduces it.

3.6.1 Preparation of CNDs Dilutions

The concentrations of CNDs which were taken for this assay included 200 µg/ml, 400 µg/ml, 600 µg/ml and 800 µg/ml. These concentrations were further diluted in methanol so that the final concentrations were between 100 µg/ml and 1000 µg/ml.

3.6.2 Preparation of Standard Solution (Ascorbic Acid)

To prepare the standard solution of AA, 1 mg of AA (Sigma Aldrich, Germany) was dissolved in 1 ml methanol. This solution was in an Eppendorf which was wrapped in aluminum foil so that it can be shielded from light. The solution was diluted using methanol so that the final concentrations were between 100 µg/ml and 1000 µg/ml. These concentrations included 200 µg/ml, 400 µg/ml, 600 µg/ml and 800 µg/ml.

3.6.3 DPPH Free-Radical Scavenging Assay

0.1mM DPPH solution was prepared in order to perform the DPPH Free-Radical Scavenging Assay. In order to do this, 3.9 mg of fresh DPPH (Sigma Aldrich, USA) was dissolved in 100ml methanol. This solution was covered in aluminum foil to protect it from the light and was kept in the dark. There were two sets of aluminum foil-covered Eppendorf tubes were labeled so that one set was for CNDs and the other was for the standard solution or ascorbic acid. 1 ml each was taken from the prepared CNDs dilutions and from the prepared ascorbic acid dilutions. These were added to the aluminum foil-covered Eppendorf tubes which had 1 ml DPPH solutions. The resulting mixtures were mixed vigorously then incubated at 37°C in the dark for 30 minutes. After this had been done, absorbance was measured at 517 nm. For this, the blank was methanol. The formula used for evaluating the percentage inhibition of DPPH radical or the percentage of scavenging radical by ascorbic acid as well as by CNDs was as follows:

$$\% \text{ scavenging radical} = [\text{Absorbance}_{(\text{control})} - \text{Absorbance}_{(\text{Test Sample})} / \text{Absorbance}_{(\text{control})}] \text{ X } 100$$

Where:

$\text{Absorbance}_{(\text{control})}$: Absorbance of the methanolic DPPH solution

$\text{Absorbance}_{(\text{test sample})}$: Absorbance of sample (ascorbic acid/CNDs) + DPPH solution

This experiment was performed in triplicates and graphs were plotted and analyzed using the Graph Pad Prism software. Through this, the antioxidant property of CNDs at different concentrations was determined.

3.7 Cytotoxicity of CNDs

The ability of CNDs to be toxic towards cancer cells was tested by checking the percentage cell viability of MCF-7 and MDA-MB-231 cells through MTT assay with CNDs. The first step towards this assay was culturing the cells which was followed by counting these cells after which the assay was performed.

3.7.1 Cell Culturing

The MCF-7 cells to be used for the cytotoxicity assay were cultured using the RPMI-1640 (Sigma Aldrich, USA) supplemented with 10% FBS (Sigma Aldrich) and 1% Penicillin and Streptomycin. The cells were added to the media and were incubated at 37°C and 5% carbon dioxide. Before each test could be performed on these cells, they were washed using the phosphate-buffered saline (PBS) (Sigma Aldrich, USA). In order to transfer the cells to a new culture media or new container, the MCF-7 cells were detached from the flask surface using trypsin-EDTA solution (Sigma Aldrich, USA). This washing was followed by re-suspending the cells into RPMI media after centrifugation at 2000 rpm for 2 minutes.

3.7.2 Cell Counting

The cells were taken from the flask in which they were cultured and were counted using a hemocytometer. This was done by first detaching the cells and then putting a small volume of media containing suspended cells on the hemocytometer slide. The four wells in the slide were named wells A, B, C and D. The cells counted in each cell were 108, 95, 89 and 92 respectively. Once the cells in each cell had been counted, the cells in the suspension per unit volume were calculated using this formula:

Cell count average = (number of cells ÷ 4) $\times$ 2 $\times$ 10^4

Or

Media required for plating = (number of squares counted $\times$ (1 $\times$ 10^4 cells/ml)) ÷ Total Counted Cells

According to this formula, the total number of cells counted were 192 $\times$ 10^4 or 1.92 $\times$ 10^6 cells/ml.

3.7.3 Drug Dilutions and Plating

96-well plates were needed for MTT Assay. One plate was required for cytotoxicity evaluation of each cell line because there were 6 dilutions of CNDs, one positive control and one negative control. The number of cells per well were kept at 10,000 and the calculation of number of cells was used to assess how much volume of the media containing cells was to be

added to each well. There were 6 dilutions for the CNDs including 0.1 µg/ml, 0.5 µg/ml, 1 µg/ml, 2.5 µg/ml, 5 µg/ml and 7.5 µg/ml. These dilutions were labeled as A, B, C, D, E and F respectively while the positive control (deionized water) and negative control (only media) were labeled G and H respectively. All of these dilutions were to be added to the 96-well plate with a volume of 200 µl in each well. Once these dilutions had been added to the plate, the plate was covered and wrapped with aluminum foil without disturbing the cells. These plates, now prepared, were put in the incubator with temperature at 37°C and carbon dioxide at 5% after labeling them with the supervisor's name, cell line and date.

3.7.4 MTT Assay

This assay was performed using the 3-(4,5-dimethyl-2-thiazolyl)-2,5,5-diphenyl-2H-tetrazolium bromide (MTT) dye. This dye is frequently used for analyses of cell proliferation and viability to check the cytotoxicity of a substance. To the prepared plates, 15 µl of MTT dye was added to each well and the plates were placed in the incubator with temperature at 37°C and carbon dioxide at 5% for 3 hours. After this the MTT solution was removed and 150 µl of DMSO was added as a solubilizing solution. This was done without disturbing the formazan crystals. The incubation was left to go on for several minutes and the solution in each well was mixed and shaken using a pipette. This led to lysis of the cells and dissolution of the crystals to spread the purple color. This plate was once again wrapped in aluminum foil before the absorption assessment could be carried out in the spectrophotometer at 550 nm wavelength.

Chapter 4

Results

4.1 Carbonization Approach for Synthesis of CNDs

CNDs were synthesized using the hydrothermal carbonization approach. This method has been reported in literature (Ortega- Liebana *et al*., 2017) and resulted in a color change which was easily detected. The AA solution which was placed inside the autoclave was translucent but the solution of CNDs after 4 hours of reaction at 250°C, followed by centrifugation and filtration was clearly yellowish brown in color. The charred black suspension in the solution was considered agglomerates which were removed through filtration. The color change aligned with the color of nanodots as reported in literature and was therefore taken as confirmation that the reaction was complete.

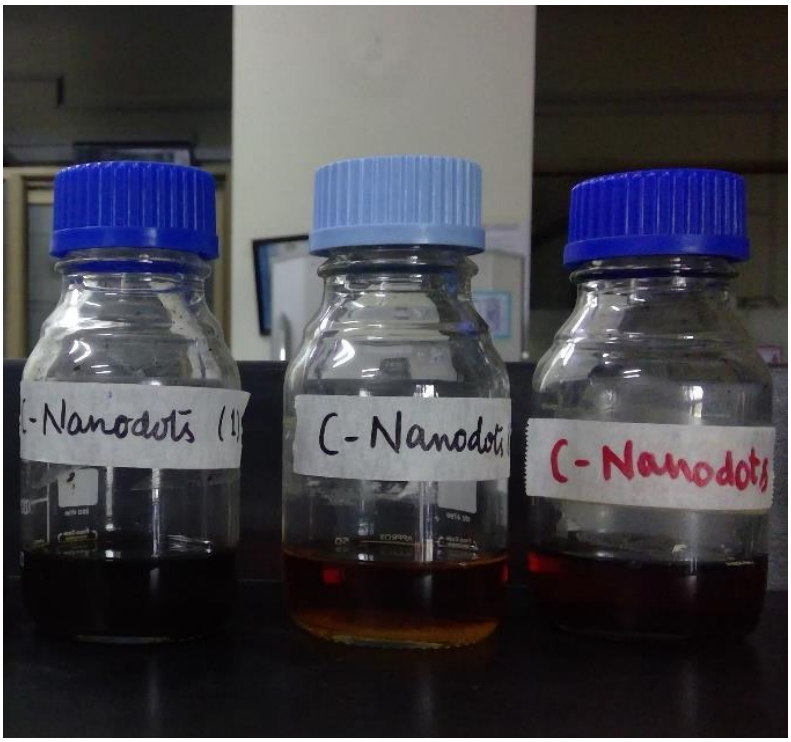

Figure 4.1 Synthesis of CNDs at 250°C for 4 hours

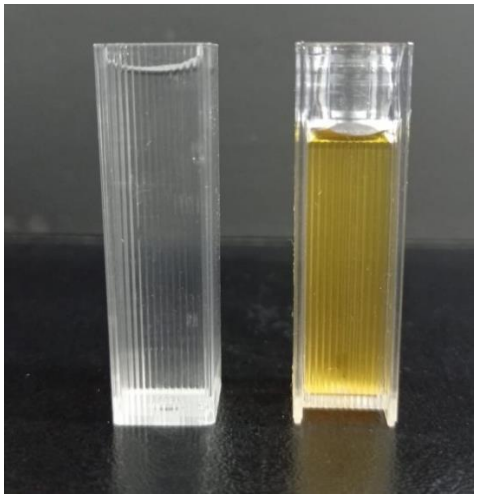

Figure 4.2 Color of CNDs after filtration

4.2 Confirmation Through 365nm UV-Lamp Excitation

Fluorescence is a defining quality of CNDs which is why it needs to be confirmed. A number of biomedical applications associated with CNDs are dependent on their fluorescence. The property of fluorescence of CNDs synthesized from AA was measured using UV-lamp excitation. A comparison of the AA solution with the CNDs solution under 365 nm UV light excitation shows that the CNDs emit fluorescence which is markedly absent from the AA solution. This fluorescence was not obtained as clearly from the concentrated solution and was observed only once the solution had been diluted. The fluorescence could be easily seen from the naked eye.

Figure 4.3 Ascorbic acid and CNDs solutions under UV excitation at 365 nm

4.3 Optical Properties of CNDs (UV-VIS Spectral Analysis)

The UV-Vis spectral analysis was used to confirm the synthesis of CNDs by observing their optical properties and comparing it with reported values from literature. Since it measures the light absorbed by the sample at certain wavelengths, the wavelength at which the highest amount of light is absorbed becomes an identifier and a defining quality of the sample. For CNDs, the absorption is normally understood to exist because of two distinct electronic transitions which happen in organic molecules and include bonding or non-bonding orbitals. The two transitions n-π^* and π-π^* are related to visible light and are therefore important with respect to absorption spectra. Out of these two, the n-π^* produces a much more profound impact on the spectrum as compared to π-π^* because it is linked with fluorescence signals and other photophysical properties. These transitions happen largely in the UV range out of which one is because of the n-π^* for C=O while the other is for π-π^* for C=C (Song *et al.*, 2017).

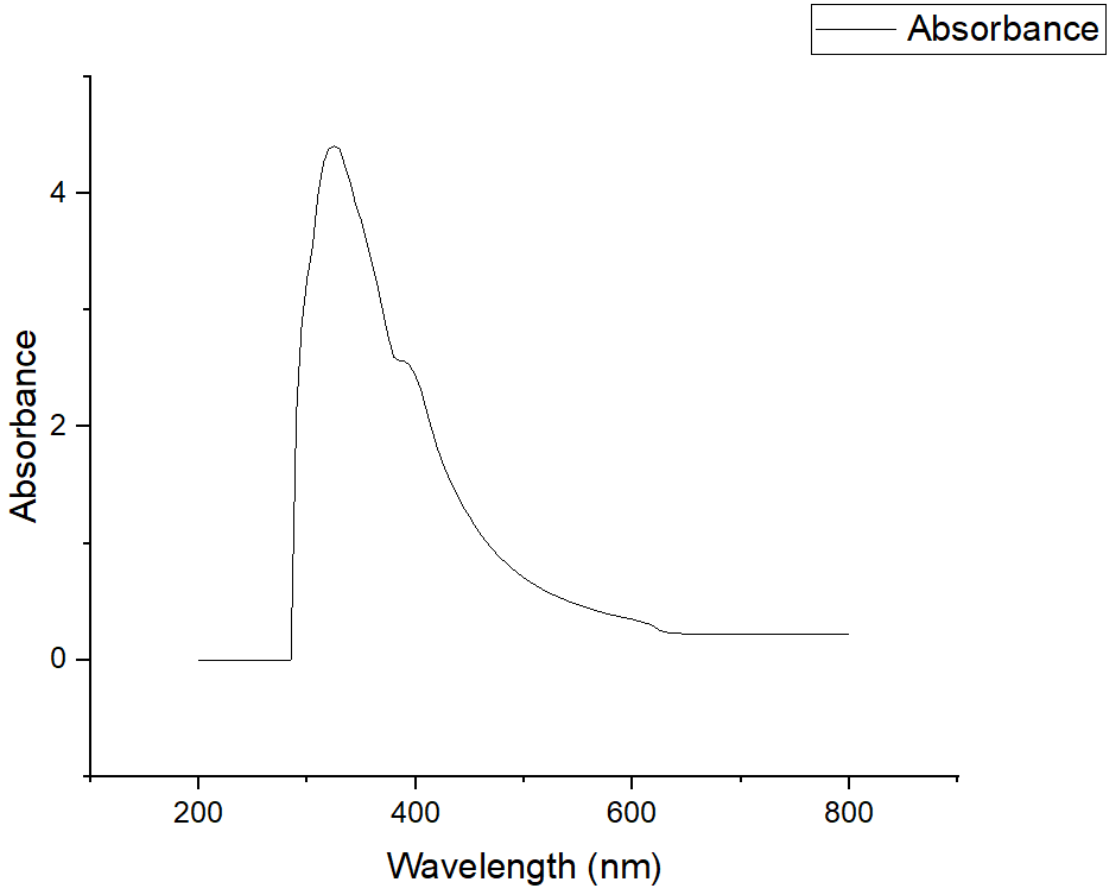

Figure 4.4 UV-Visible spectrum of absorption of AA (ascorbic acid) CNDs

In the results obtained for the AA CNDs, the wavelength range for observation was set between 200 nm and 800 nm. This first transition can be seen in Figure 4.4 at the main peak

at around 320 nm while the other transition can be seen at the small projection at around 400 nm. The spike in absorbance is seen to be fully located in the UV range while the tail of the absorbance curve is present in the visible range.

4.4 Characterization of CNDs

4.4.1 SEM Analysis of CNDs

Scanning Electron Microscopy is the technique used to analyze the morphology and size of nanoparticles. In the case of CNDs, their size and morphology determine their future use and biomedical applications. Therefore, SEM analysis was performed at voltage 20kV and magnification at 50,000 to visualize the nanodots. Images obtained from the SEM machine confirm that the nanodots have a spherical morphology and the sizes are all in the nano range. Moreover, some particles are still agglomerated despite the ultrasonication that they were previously exposed to.

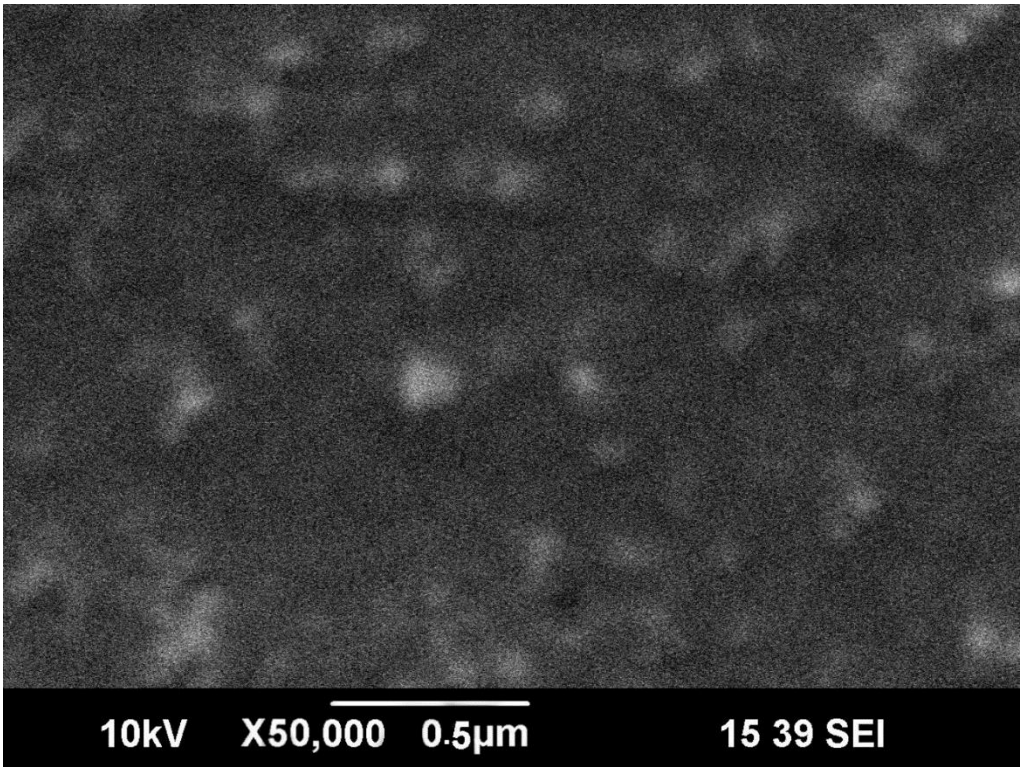

Figure 4.5 SEM image showing spherical morphology and nano range size of CNDs

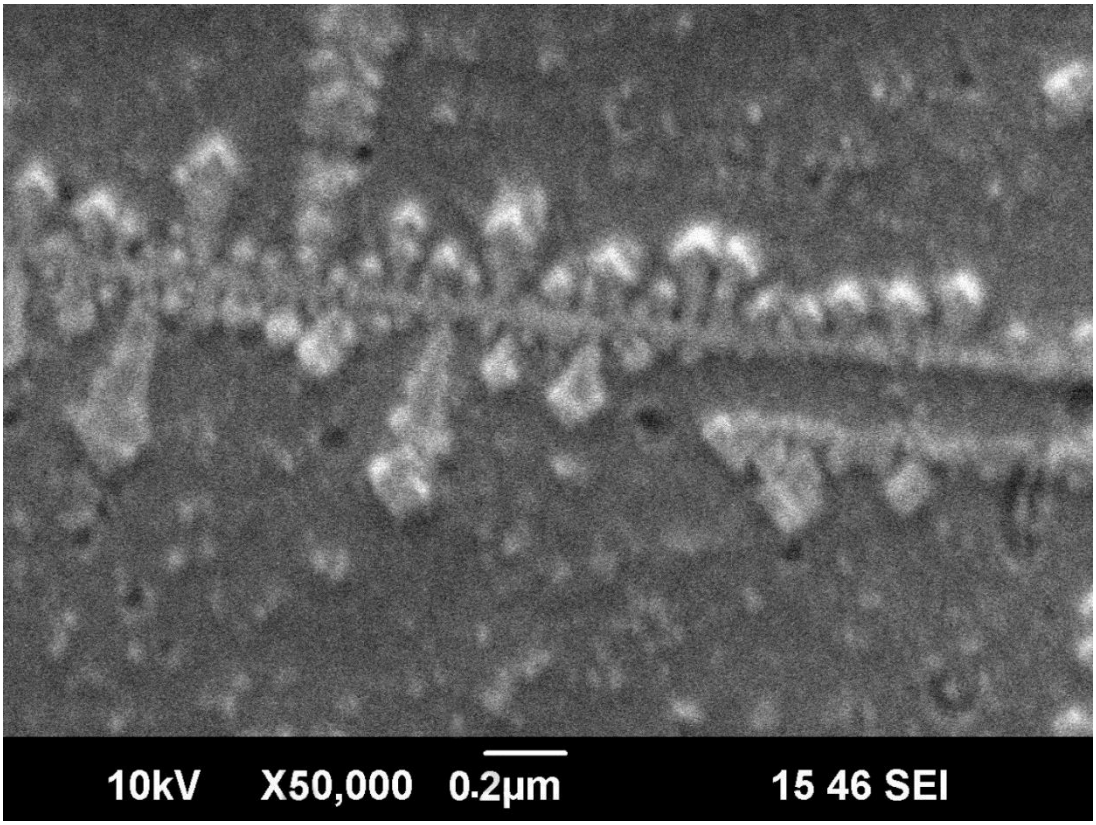

Figure 4.6 SEM analysis showing that some nanodots are agglomerated

4.4.2 X-ray Diffraction Analysis

X-ray Diffraction Analysis or XRD analysis is a crystallography technique used to reveal and ascertain the crystal structure of a sample. In the case of CNDs, their XRD analysis will reveal details about their crystalline structure and properties. The reported results for the XRD analysis in Figure 4.7 show that there are two easily discernible peaks for CNDs. One of these peaks can be detected when the value of 2θ is around 22°. This peak is for the 002 diffraction pattern as reported in literature (Gong *et al.*, 2014). The other peak can be seen where the value of 2θ is around 42°. This second less clear peak corresponds to the 100 diffraction pattern. The first peak is because of graphitic carbon while the other is for graphene like structures. These peaks confirm that the carbon present in these CNDs is in amorphous structure because the carbon atoms in the sample are highly disarrayed.

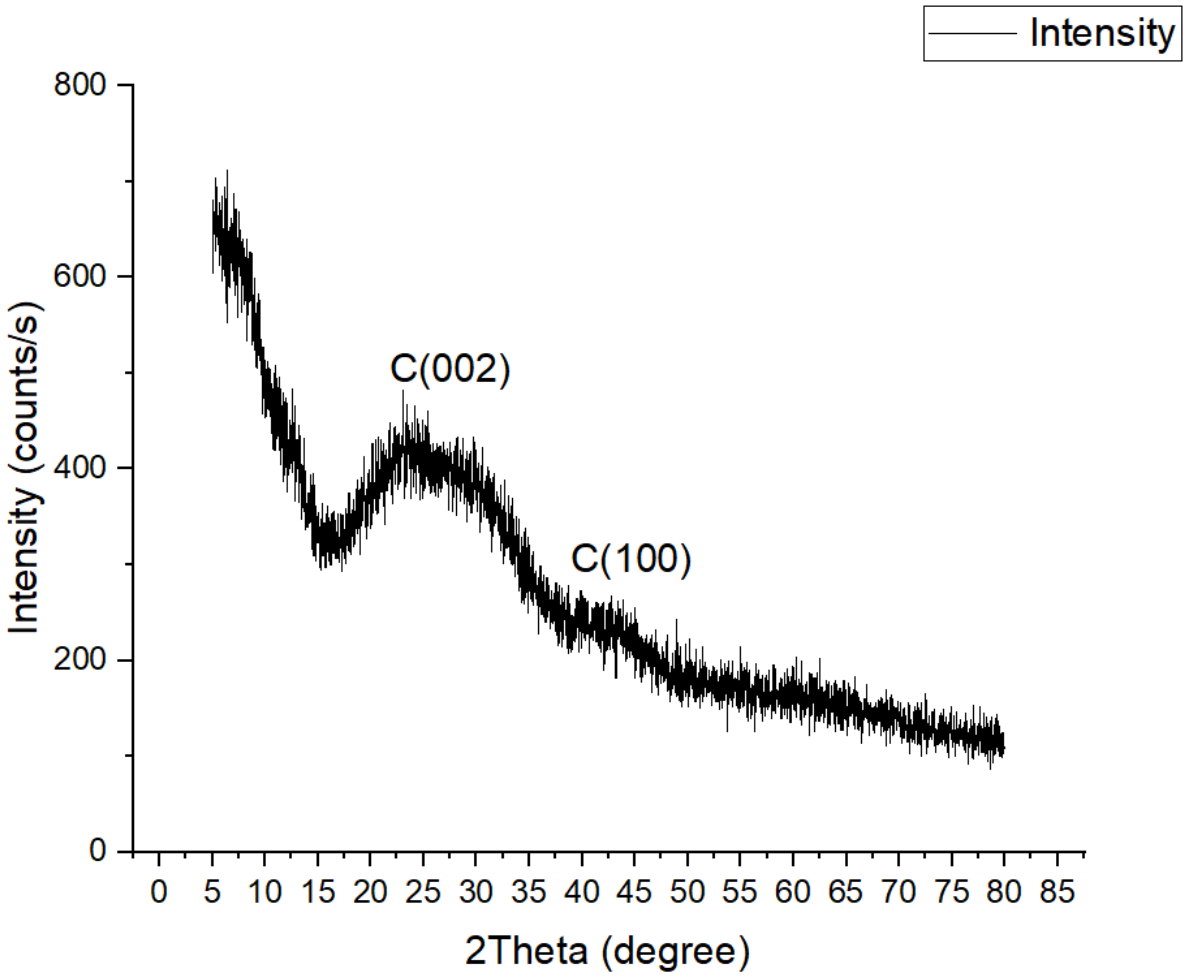

Figure 4.7 XRD analysis of AA (ascorbic acid) CNDs

4.4.3 Fourier Transformed Infrared Spectroscopy (FTIR)

Fourier Transformed Infrared Spectroscopy (FTIR) analysis confirmed the functional groups which are present on the surface of the CNDs. Figure 4.8 shows the FTIR analysis when performed on the CNDs sample and this analysis confirms that a number of functional groups are definitively present on the surface of these CNDs synthesized from ascorbic acid. The first discernable peak at around 3400 cm^{-1} corresponds to the O-H bond and shows that there is an OH functional group present at the surface of these CNDs. The second prominent peak is at around 1600 cm^{-1} which is composed of two peaks and may correspond to both a C=O bond and a triple covalent bond between two C atoms. Therefore, this peak shows that there is an aldehydic or carboxylic group present on the surface of these carbon atoms. The C-H

and C-O bonds shown at the peaks around 1100 cm^{-1} show that the methyl and ketonic or carboxylic groups are also present on the surface of the synthesized CNDs.

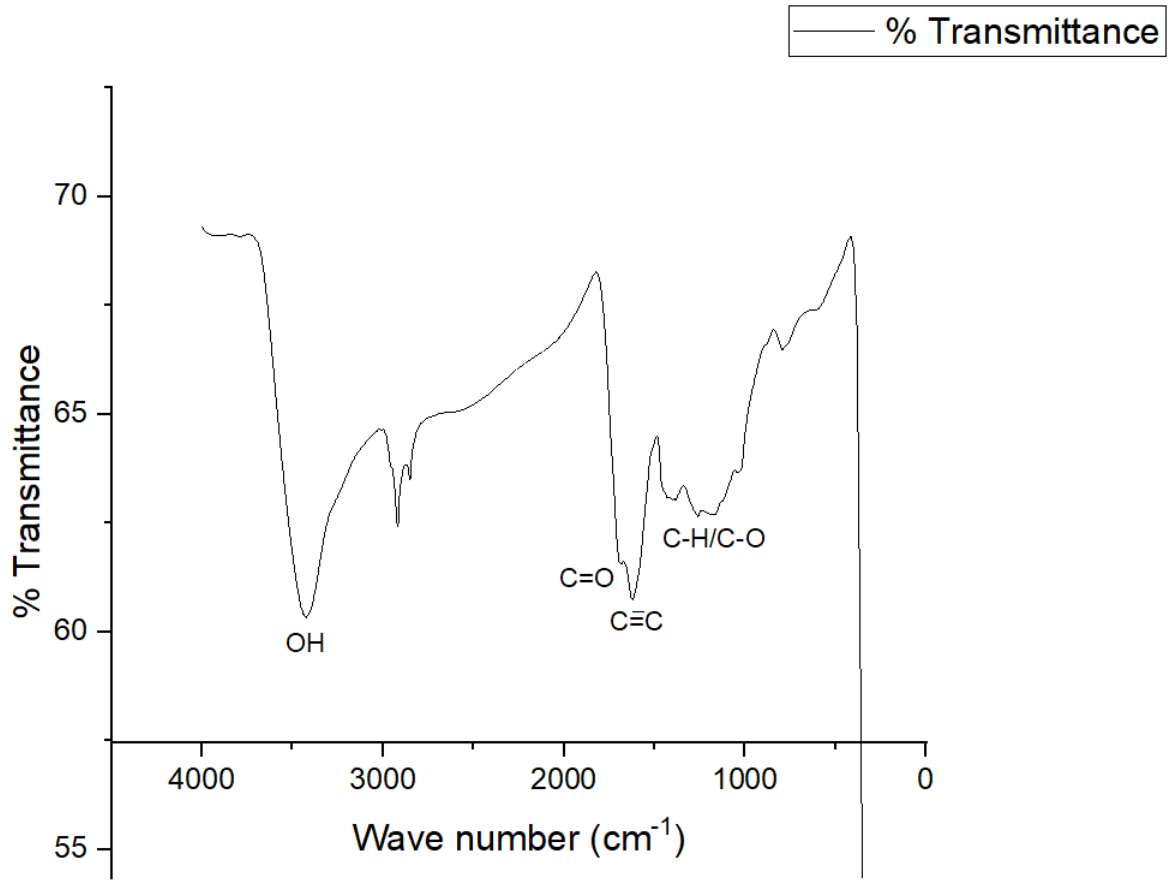

Figure 4.8 FTIR analysis of AA (ascorbic acid) CNDs

4.5 Antibacterial Activity of CNDs

CNDs exhibit antibacterial activity which can be comparable to the positive controls or antibiotics including Cefotaxime (CTX) (Oxoid Ltd., UK) and Ciprofloxacin (CIP) (Oxoid Ltd., UK). Results obtained from comparing the three concentrations of CNDs, 35 µg/ml, 45 µg/ml and 55 µg/ml, confirm this. CNDs were more effective against *Escherichia coli* and *Staphylococcus aureus* but less so for *Klebsiella pneumonia*. However, this variation aligned with a similar variation shown for both antibiotics. The zones of inhibition for all three bacterial strains increased in diameter as the concentration of CNDs rose from 35 µg/ml to 45 µg/ml and then to 55 µg/ml. This showed that the antibacterial property of the CNDs

increased with increase in concentration. Negative control (deionized water) did not show any zone of inhibition as expected which confirmed the integrity of the experiment.

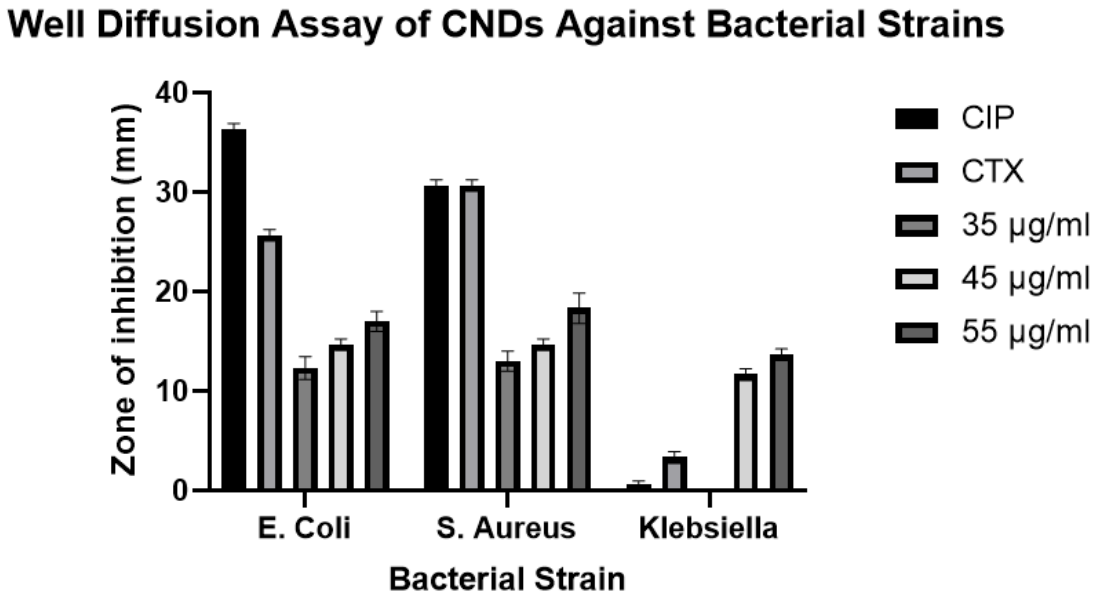

Figure 4.9 Comparison of zones of inhibition of bacterial isolates after the application of CNDs of various concentrations

4.6 Antioxidant Assay

The potential of CNDs to have a free radical scavenging ability was calculated using the DPPH inhibition assay. This was done for four concentrations of CNDs including 200 µg/ml, 400 µg/ml, 600 µg/ml and 800 µg/ml. Similar four concentrations of ascorbic acid were used for comparison as the positive control because it is a natural antioxidant. The results from this assay confirm that the free radical scavenging ability of CNDs increases when the concentration increases. They also show that the free radical scavenging ability of CNDs is considerably less than ascorbic acid at lower concentrations but that the gap narrows once the concentrations rise. At 200 µg/ml, the difference is as high as 40% which then closes down to around 10% at 800 µg/ml. The huge difference at smaller concentrations, however, shows that even though CNDs have good antioxidant ability, it is not similar or even close to the antioxidant ability of Ascorbic Acid.

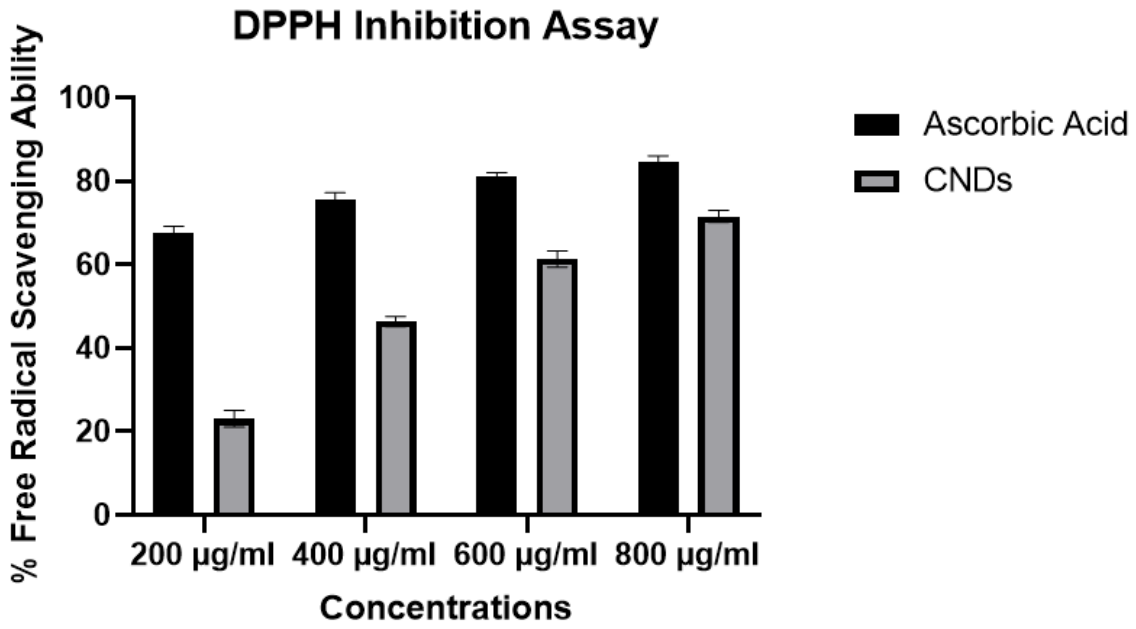

Figure 4.10 Free radical scavenging ability through DPPH inhibition of CNDs compared with that of Ascorbic Acid

4.7 Cytotoxicity Assay

The MTT assay was used to assess the cytotoxicity of the synthesized CNDs. The lack of cytotoxicity of CNDs is reported in the previous literature. (Sivasankarapillai, 2020) This biocompatibility of CNDs was confirmed by adding six concentrations of CNDs to both MCF-7 and MDA-MB-231 cells. These six concentrations were 0.1 µg/ml, 0.5 µg/ml, 1 µg/ml, 2.5 µg/ml, 5 µg/ml and 7.5 µg/ml. The results obtained for MCF-7 showed that the cell viability is a hundred percent when the concentration is 0 showing the control. This cell viability very gradually dropped only a few percentages over the course of the six concentrations. As increasing concentrations showed decreasing cell viability, it showed that the cytotoxicity of CNDs could rise at higher concentrations but at low concentrations, they were not toxic. The same pattern was observed for both cell lines as for MDA-MB-231 as well, the percentage viability slowly dropped a few percentages over the six dilutions but the overall impact was negligible.

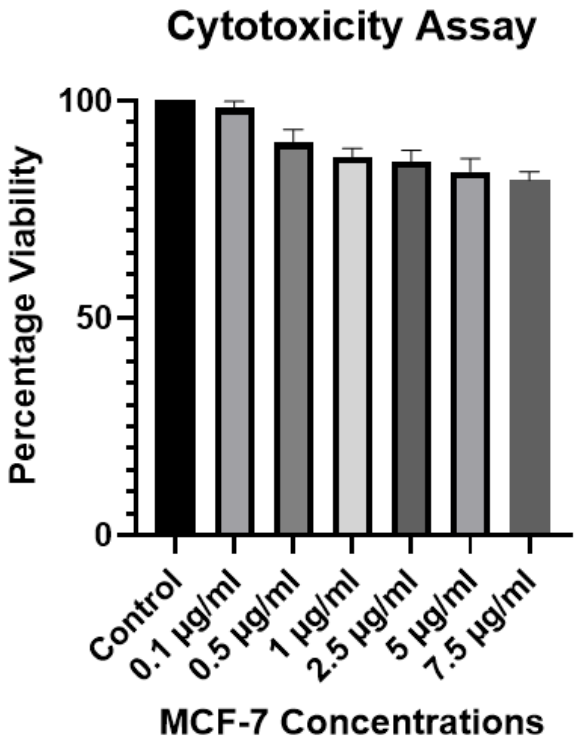

Figure 4.11 Percentage viability for MCF-7 cell line with different concentrations of CNDs

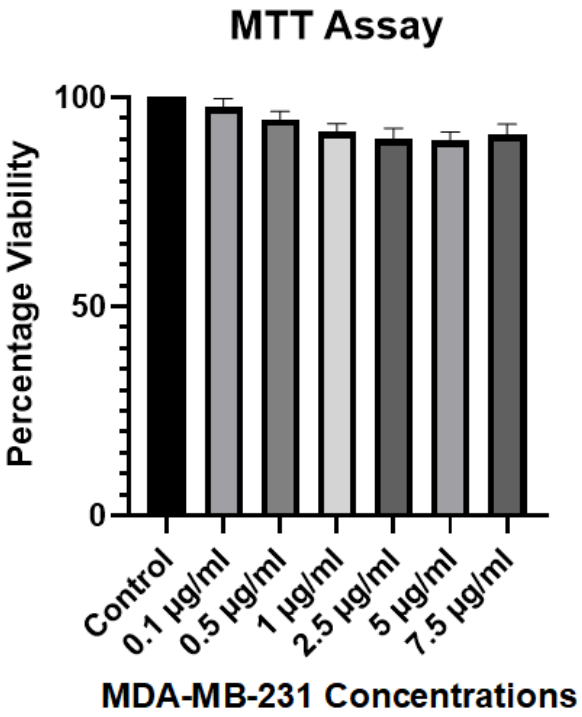

Figure 4.12 Percentage viability for MDA-MB-231 cells with different CND concentrations

Chapter 5

DISCUSSION

Applications of nanotechnology are based on the idea that nanoparticles within the standard size range of 0.1 to 100nm have chemical and physical properties which are distinct from properties held by the same matter at a larger size (Idrees, 2015). Optical, electronic, magnetic, mechanical and thermal properties are especially exploited for a range of applications in a large number of industrial fields (Nikalje, 2015). CNDs are one such example of nanoparticles exploited for their unique properties. In the case of CNDs, these properties are primarily optical as the ability of CNDs to fluoresce and to have bioluminescence (Xu *et al*., 2018). The zero-dimensional morphology, homogeneity and surface passivation of CNDs are made even better by the fact that CNDs are biocompatible hence exhibiting no toxicity inside a biological system (Cayuela *et al*., 2016). Because of these properties, the application of CNDs in the field of biomedicine are wide in range. Use of CNDs *in vivo* is especially enhanced through these features (Sivasankarapillai *et al*., 2020).

These CNDs can be synthesized in a number of different ways which make research on CNDs feasible as a synthesis method suited to laboratory environment and available resources can be picked for use. Electrochemical method includes electrochemical carbonization using electrodes (Bao *et al*., 2011). Pyrolysis can also be done inside a microwave to carbonize a chemical or organic carbon source which can then undergo surface passivation (de Medeiros *et al*., 2019). For ultrasonic methods, ultrasonic waves carry out the carbonization of the source to create CNDs (Ma *et al*., 2012). Laser ablation method can also be used for synthesizing CNDs in which a laser is aimed at a solid carbon source and this interaction between the two produces nanoparticles (Amendola and Meneghetti, 2013).

However, out of all the methods of synthesis of CNDs, the hydrothermal method is the most frequently used method because of the high fluorescence and size homogeneity achieved through this method (Zhang *et al*., 2018). This method involves a hydrothermal autoclave having the chemical source for carbon sealed inside it which is then placed on a certain high temperature for an extended period of time until the source carbonizes and the nanodots are produced (Einarsrud and Grande, 2014). This study also produced CNDs using the hydrothermal method of synthesis using a Teflon-lined hydrothermal autoclave. The results of this synthesis aligned with the expected physical and colorimetric features of CNDs. A

solution containing CNDs must be brownish yellow while the ascorbic acid solution is colorless and translucent (Ortega-Liebana *et al*., 2017). The CNDs solution produced in this study also had a brownish yellow color and a burnt smell which confirmed that carbonization of AA had successfully taken place inside the hydrothermal autoclave. CNDs produced in the study were also found to give off a bright blue fluorescence when excited by ultraviolet wavelength 365nm. This was also consistent with research on CNDs which give off maximum fluorescence when excited at the UV wavelength of 365nm which is soft and blue in color (Nawaz *et al*., 2013). Optical properties of the CNDs determined through UV-Vis spectral analysis also revealed that CNDs were present because of the peaks obtained as a result. These peaks corresponded with the electronic transitions that are characteristic of CNDs as they were n-π^* and π-π^* with the former being more profound and easily discernible while the latter being smaller and more difficult to identify (Song *et al*., 2017). Both the color and the fluorescence properties therefore confirmed the synthesis of CNDs from the hydrothermal synthesis while subsequent analyses were carried out to characterize the nanodots.

For characterization, the first step in the study was carrying out a SEM analysis through which the size and morphology of the synthesized CNDs was determined. CNDs are supposed to be spherical in shape and range around a size as small as 20nm (Cailotto *et al*., 2018). Results obtained from the SEM analysis in this study showed a size and morphology which was compatible with this range. Moreover, these images also showed that some of the nanoparticles were in the form of agglomerates which is also continuous with the trend of agglutinizing during carbonization inside a hydrothermal autoclave. Even though filtration, centrifugation and sonication is meant to break up and separate agglomerates, the images show that some agglomerates were still left behind for the SEM analysis to pick up. This was followed by an XRD analysis in the study through which the crystalline structure of the CNDs were observed. Results obtained in the study showed that there were two clear peaks in the spectrum. The first peak where the value of 2θ is at 22° was confirmed by literature to be for the 002 diffraction pattern while the other one with 2θ at 42° was understood to be for the 100 diffraction (Gong *et al*., 2014). These peaks show that there is disarray in the CNDs and that they are actually in amorphous form. The next step in the study was FTIR through which the different functional groups were analyzed. The peak around 3400 cm^{-1} was supposed to be for the O-H bond while the one at 1600 cm^{-1} showed the double and triple covalent bonds between two carbon atoms on the surface of CNDs and another one at 1100 cm^{-1} was about

C-H and C-O bonds. These two bonds between carbon atoms aligned with aldehydic or carboxylic functional groups while the bonds between carbon and hydrogen and carbon and oxygen showed that methyl or carboxylic bonds were also present. Ketonic groups could also be deduced from these results. These results align with the FTIR results obtained for CNDs in literature (Cailotto *et al*., 2018).

Once characterization was done, the study moved on to assessing biological applications of CNDs. For this purpose, the nanodots were checked to see if they had any antimicrobial activity. Literature confirms that excited CNDs are capable of producing reactive oxygen species (ROS) which leads to them killing microbes (Dong *et al*., 2020). For this purpose, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* strains were used, and the inhibition zones produced by nanodots were compared with antibiotics to assess their antimicrobial activity. It was discovered that the nanodots were especially effective against *Escherichia coli* and *Staphylococcus aureus* as a rise in concentration of CNDs increased the diameters of their zones of inhibition for both of these strains in a linear manner. This impact was noticed to not be that prominent for *Klebsiella pneumonia* where inhibition existed at higher concentrations but was significantly small compared to the other two strains. To connect the ability of excited CNDs with the ability to produce ROS, the ability of CNDs to scavenge free radicals was also assessed in the study. Literature showed that CNDs have good antioxidant capability which can be assessed using the DPPH assay and can be high enough to be compared to that of AA which is used as standard (Ruiz *et al*., 2017). This antioxidant assay using DPPH revealed that CNDs possessed considerable ability to scavenge free radicals which was even comparable to AA (standard) at higher concentrations.

The most important application for CNDs which was checked in this study was the cytotoxicity of synthesized CNDs which was done using MTT assay. This was especially critical because the biocompatibility of CNDs is one of their defining features and is crucial in all their biological applications carried out *in vivo* (Gong *et al*., 2014). CNDs were assessed for their biocompatibility by checking whether they had any cytotoxic impact on cancer cell lines like MCF-7 and MDA-MB-231. Literature has showed that CNDs at small and progressively higher concentrations have next to no cytotoxic impact on either of these cell lines (Muktha *et al*., 2020). The percentages of cell viability obtained from this study also confirmed that the viability was almost 100% for rising concentrations of CNDs and it hardly ever dipped below 90% for both MCF-7 and MDA-MB-231 even at high concentrations like 7.5µg/ml. This showed that the same concentrations at which CNDs were capable of

producing fluorescence and bioluminescence did not have any toxic impact on the cells. It also showed that the synthesized CNDs were biocompatible and therefore eligible for further applications in biomedicine. The applications in the field of biomedicine possible for these CNDs are dependent on the properties of fluorescence and biocompatibility. These can lead to applications in the field of bioimaging which can be of use in the diagnostics field, primarily in the field of oncology. Since the CNDs have no impact on cell viability, CNDs can also be conjugated with antibodies for detection and with drugs for targeted delivery. Because of these properties, CNDs can also be passivated to detect certain chemical compounds *in vivo*. These applications show that the range for CNDs is wide and considerable.

CONCLUSION AND FUTURE PROSPECTS

This current study was focused on synthesizing CNDs from ascorbic acid, a chemical source, through the hydrothermal process. Synthesis was done by leaving the carbon source sealed in a Teflon-lined hydrothermal autoclave at 250°C for four hours before filtration and purification of the supernatant. This was followed by characterization and determination of properties which confirmed that the CNDs synthesized in this study aligned with the physicochemical characteristics CNDs described in literature. For this purpose, UV-Vis spectral analysis, XRD spectroscopy, SEM and FTIR were carried out on the CNDs. Their fluorescence was confirmed, and biocompatibility ensured. An estimate of their antimicrobial and antioxidant activities was also taken in order to round off the properties of the synthesized CNDs.

Properties like antimicrobial activity, antioxidant activity and cytotoxicity were chosen because these were understood to be integral in putting these CNDs to use, especially in applications concerning the field of biomedicine. Future prospects of these CNDs therefore heavily circulate around applications of the nanodots synthesized and characterized in this study. Considering the fact that the fluorescence and biocompatibility of CNDs got confirmed in this study, the applications which must be explored in further research should primarily include bioimaging and the possible diagnostic properties of CNDs. This research can discover the ways in which conjugation with a tag can lead to the CNDs being used as the perfect candidate for *in vivo* bioimaging. Other than this, further research can also focus on the exact pathways followed by the CNDs which leads to them being biocompatible and also leaves them capable of entering cells to exhibit fluorescence from inside cells. This research can be integral in biomedicine, especially oncology, where breakthroughs in early diagnosis are critically needed.

REFERENCES

Alexis, F., Pridgen, E., Molnar, L. K., & Farokhzad, O. C. (2008). Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Molecular pharmaceutics*, *5*(4), 505-515.

Amendola, V., & Meneghetti, M. (2013). What controls the composition and the structure of nanomaterials generated by laser ablation in liquid solution?. *Physical Chemistry Chemical Physics*, *15*(9), 3027-3046.

Anwar, S., Ding, H., Xu, M., Hu, X., Li, Z., Wang, J., ... & Bi, H. (2019). Recent advances in synthesis, optical properties, and biomedical applications of carbon dots. *ACS Applied Bio Materials*, *2*(6), 2317-2338.

Archakov, A. I. (2010). Nanobiotechnologies in medicine: nanodiagnostics and nanodrugs. *Biochemistry (Biokhimiya). Supplemental Series B, Biomedical Chemistry*, *4*(1), 2.

Asghari, F., Jahanshiri, Z., Imani, M., Shams-Ghahfarokhi, M., & Razzaghi-Abyaneh, M. (2016). Antifungal nanomaterials: synthesis, properties, and applications. In *Nanobiomaterials in antimicrobial therapy* (pp. 343-383). William Andrew Publishing.

Baker, S. N., & Baker, G. A. (2010). Luminescent carbon nanodots: emergent nanolights. *Angewandte Chemie International Edition*, *49*(38), 6726-6744.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, *6*(2), 71-79.

Bao, L., Zhang, Z. L., Tian, Z. Q., Zhang, L., Liu, C., Lin, Y., ... & Pang, D. W. (2011). Electrochemical tuning of luminescent carbon nanodots: from preparation to luminescence mechanism. *Advanced materials*, *23*(48), 5801-5806.

Battaglia, S., Evangelisti, S., Leininger, T., Pirani, F., & Faginas-Lago, N. (2020). A novel intermolecular potential to describe the interaction between the azide anion and carbon nanotubes. *Diamond and Related Materials*, *101*, 107533.

Bawage, S. S., Tiwari, P. M., Pillai, S., Dennis, V., & Singh, S. R. (2013). Recent advances in diagnosis, prevention, and treatment of human respiratory syncytial virus. *Advances in virology*, *2013*.

Bello, S. A., Agunsoye, J. O., & Hassan, S. B. (2015). Synthesis of coconut shell nanoparticles via a top down approach: Assessment of milling duration on the particle sizes and morphologies of coconut shell nanoparticles. *Materials Letters*, *159*, 514-519.

Bhattacharyya, S., Ehrat, F., Urban, P., Teves, R., Wyrwich, R., Döblinger, M., ... & Stolarczyk, J. K. (2017). Effect of nitrogen atom positioning on the trade-off between emissive and photocatalytic properties of carbon dots. *Nature communications*, *8*(1), 1-9.

Bidard, F. C., Proudhon, C., & Pierga, J. Y. (2016). Circulating tumor cells in breast cancer. *Molecular oncology*, *10*(3), 418-430.

Bourlinos, A. B., Stassinopoulos, A., Anglos, D., Zboril, R., Karakassides, M., & Giannelis, E. P. (2008). Surface functionalized carbogenic quantum dots. *small*, *4*(4), 455-458.

Bulfoni, M., Gerratana, L., Del Ben, F., Marzinotto, S., Sorrentino, M., Turetta, M., ... & Cesselli, D. (2016). In patients with metastatic breast cancer the identification of circulating tumor cells in epithelial-to-mesenchymal transition is associated with a poor prognosis. *Breast Cancer Research*, *18*(1), 1-15.

Cailotto, S., Amadio, E., Facchin, M., Selva, M., Pontoglio, E., Rizzolio, F., ... & Perosa, A. (2018). Carbon dots from sugars and ascorbic acid: role of the precursors on morphology, properties, toxicity, and drug uptake. *ACS medicinal chemistry letters*, *9*(8), 832-837.

Cao, L., Sahu, S., Anilkumar, P., Bunker, C. E., Xu, J., Fernando, K. S., ... & Sun, Y. P. (2011). Carbon nanoparticles as visible-light photocatalysts for efficient CO2 conversion and beyond. *Journal of the American Chemical Society*, *133*(13), 4754-4757.

Cao, Y. C., Jin, R., & Mirkin, C. A. (2002). Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection. *Science*, *297*(5586), 1536-1540.

Cayuela, A., Soriano, M. L., & Valcárcel, M. (2015). Photoluminescent carbon dot sensor for carboxylated multiwalled carbon nanotube detection in river water. *Sensors and Actuators B: Chemical*, *207*, 596-601.

Chandra, A., Deshpande, S., Shinde, D. B., Pillai, V. K., & Singh, N. (2014). Mitigating the cytotoxicity of graphene quantum dots and enhancing their applications in bioimaging and drug delivery. *ACS Macro Letters*, *3*(10), 1064-1068.

Chen, B. B., Liu, M. L., Li, C. M., & Huang, C. Z. (2019). Fluorescent carbon dots functionalization. *Advances in colloid and interface science*, *270*, 165-190.

da Silva, J. C. E., & Gonçalves, H. M. (2011). Analytical and bioanalytical applications of carbon dots. *TrAC Trends in Analytical Chemistry*, *30*(8), 1327-1336.

Das, R., Bandyopadhyay, R., & Pramanik, P. (2018). Carbon quantum dots from natural resource: A review. *Materials today chemistry*, *8*, 96-109.

Das, R. K., & Mohapatra, S. (2017). Highly luminescent, heteroatom-doped carbon quantum dots for ultrasensitive sensing of glucosamine and targeted imaging of liver cancer cells. *Journal of Materials Chemistry B*, *5*(11), 2190-2197.

de Medeiros, T. V., Manioudakis, J., Noun, F., Macairan, J. R., Victoria, F., & Naccache, R. (2019). Microwave-assisted synthesis of carbon dots and their applications. *Journal of Materials Chemistry C*, *7*(24), 7175-7195.

De, B., & Karak, N. (2017). Recent progress in carbon dot–metal based nanohybrids for photochemical and electrochemical applications. *Journal of Materials Chemistry A*, *5*(5), 1826-1859.

Deng, J., Lu, Q., Mi, N., Li, H., Liu, M., Xu, M., ... & Yao, S. (2014). Electrochemical synthesis of carbon nanodots directly from alcohols. *Chemistry–A European Journal*, *20*(17), 4993-4999.

Ding, Y., Zhang, F., Xu, J., Miao, Y., Yang, Y., Liu, X., & Xu, B. (2017). Synthesis of short-chain passivated carbon quantum dots as the light emitting layer towards electroluminescence. *RSC advances*, *7*(46), 28754-28762.

Dong, X., Liang, W., Meziani, M. J., Sun, Y. P., & Yang, L. (2020). Carbon dots as potent antimicrobial agents. *Theranostics*, *10*(2), 671.

Dreaden, E. C., Alkilany, A. M., Huang, X., Murphy, C. J., & El-Sayed, M. A. (2012). The golden age: gold nanoparticles for biomedicine. *Chemical Society Reviews*, *41*(7), 2740-2779.

Einarsrud, M. A., & Grande, T. (2014). 1D oxide nanostructures from chemical solutions. *Chemical Society Reviews*, *43*(7), 2187-2199.

Eustis, S., & El-Sayed, M. A. (2006). Why gold nanoparticles are more precious than pretty gold: noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes. *Chemical society reviews*, *35*(3), 209-217.

Feng, H., & Qian, Z. (2018). Functional carbon quantum dots: A versatile platform for chemosensing and biosensing. *The Chemical Record*, *18*(5), 491-505.

Galanzha, E. I., Shashkov, E. V., Kelly, T., Kim, J. W., Yang, L., & Zharov, V. P. (2009). In vivo magnetic enrichment and multiplex photoacoustic detection of circulating tumour cells. *Nature nanotechnology*, *4*(12), 855-860.

Gao, G., Jiang, Y. W., Jia, H. R., Yang, J., & Wu, F. G. (2018). On-off-on fluorescent nanosensor for Fe3+ detection and cancer/normal cell differentiation via silicon-doped carbon quantum dots. *Carbon*, *134*, 232-243.

Gascón, V., Jiménez, M. B., Blanco, R. M., & Sanchez-Sanchez, M. (2018). Semi-crystalline Fe-BTC MOF material as an efficient support for enzyme immobilization. *Catalysis Today*, *304*, 119-126.

Ge, J., Jia, Q., Liu, W., Guo, L., Liu, Q., Lan, M., ... & Wang, P. (2015). Red- emissive carbon dots for fluorescent, photoacoustic, and thermal theranostics in living mice. *Advanced Materials*, *27*(28), 4169-4177.

Ghafary, S. M., Nikkhah, M., Hatamie, S., & Hosseinkhani, S. (2017). Simultaneous gene delivery and tracking through preparation of photo-luminescent nanoparticles based on graphene quantum dots and chimeric peptides. *Scientific reports*, *7*(1), 1-14.

Gong, J., An, X., & Yan, X. (2014). A novel rapid and green synthesis of highly luminescent carbon dots with good biocompatibility for cell imaging. *New Journal of Chemistry*, *38*(4), 1376-1379.

Guo, D., Xie, G., & Luo, J. (2013). Mechanical properties of nanoparticles: basics and applications. *Journal of physics D: applied physics*, *47*(1), 013001.

Hu, L., Sun, Y., Li, S., Wang, X., Hu, K., Wang, L., ... & Wu, Y. (2014). Multifunctional carbon dots with high quantum yield for imaging and gene delivery. *Carbon*, *67*, 508-513.

Hu, S., Liu, J., Yang, J., Wang, Y., & Cao, S. (2011). Laser synthesis and size tailor of carbon quantum dots. *Journal of Nanoparticle Research*, *13*(12), 7247-7252.

Hu, Y., Yang, J., Jia, L., & Yu, J. S. (2015). Ethanol in aqueous hydrogen peroxide solution: hydrothermal synthesis of highly photoluminescent carbon dots as multifunctional nanosensors. *Carbon*, *93*, 999-1007.

Hur, J. U., Han, J. S., Shin, J. R., Park, H. Y., Choi, S. C., Jung, Y. G., & An, G. S. (2019). Fabrication of SnO2-decorated Fe3O4 nanoparticles with anionic surface modification. *Ceramics International*, *45*(17), 21395-21400.

Hutton, G. A., Martindale, B. C., & Reisner, E. (2017). Carbon dots as photosensitisers for solar-driven catalysis. *Chemical Society Reviews*, *46*(20), 6111-6123.

Idrees, M. (2015). Role of nanotechnology in medical sciences: a review. *Int. J. Drug Discovery*, *5*, 14-24.

Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green Chemistry*, *13*(10), 2638-2650.

Janni, W. J., Rack, B., Terstappen, L. W., Pierga, J. Y., Taran, F. A., Fehm, T., ... & Lucci, A. (2016). Pooled analysis of the prognostic relevance of circulating tumor cells in primary breast cancer. *Clinical cancer research*, *22*(10), 2583-2593.

Jhonsi, M. A., Ananth, D. A., Nambirajan, G., Sivasudha, T., Yamini, R., Bera, S., & Kathiravan, A. (2018). Antimicrobial activity, cytotoxicity and DNA binding studies of carbon dots. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *196*, 295-302.

Kairdolf, B. A., Smith, A. M., Stokes, T. H., Wang, M. D., Young, A. N., & Nie, S. (2013). Semiconductor quantum dots for bioimaging and biodiagnostic applications. *Annual review of analytical chemistry*, *6*, 143-162.

Kelarakis, A. (2014). From highly graphitic to amorphous carbon dots: A critical review. *MRS Energy & Sustainability*, *1*.

Khan, M. S., Pandey, S., Talib, A., Bhaisare, M. L., & Wu, H. F. (2015). Controlled delivery of dopamine hydrochloride using surface modified carbon dots for neuro diseases. *Colloids and Surfaces B: Biointerfaces*, *134*, 140-146.

Kim, C., & Lee, H. (2018). Light-assisted surface reactions on metal nanoparticles. *Catalysis Science & Technology*, *8*(15), 3718-3727.

Kosmala, A., Wright, R., Zhang, Q., & Kirby, P. (2011). Synthesis of silver nano particles and fabrication of aqueous Ag inks for inkjet printing. *Materials Chemistry and Physics*, *129*(3), 1075-1080.

Kot, M., Major, Ł., Lackner, J. M., Chronowska-Przywara, K., Janusz, M., & Rakowski, W. (2016). Mechanical and tribological properties of carbon-based graded coatings. *Journal of Nanomaterials*, *2016*.

Kwan, T. T., Bardia, A., Spring, L. M., Giobbie-Hurder, A., Kalinich, M., Dubash, T., ... & Maheswaran, S. (2018). A digital RNA signature of circulating tumor cells predicting early therapeutic response in localized and metastatic breast cancer. *Cancer discovery*, *8*(10), 1286-1299.

Lambert, A. W., Pattabiraman, D. R., & Weinberg, R. A. (2017). Emerging biological principles of metastasis. *Cell*, *168*(4), 670-691.

Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Vander Elst, L., & Muller, R. N. (2008). Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chemical reviews*, *108*(6), 2064-2110.

LeCroy, G. E., Sonkar, S. K., Yang, F., Veca, L. M., Wang, P., Tackett, K. N., ... & Sun, Y. P. (2014). Toward structurally defined carbon dots as ultracompact fluorescent probes. *ACS nano*, *8*(5), 4522-4529.

Lee, S., Choi, S. S., Li, S. A., & Eastman, J. A. (1999). Measuring thermal conductivity of fluids containing oxide nanoparticles. *Journal of Heat Transfer*, 280-285.

Lei, Z., Xu, S., Wan, J., & Wu, P. (2016). Facile synthesis of N-rich carbon quantum dots by spontaneous polymerization and incision of solvents as efficient bioimaging probes

and advanced electrocatalysts for oxygen reduction reaction. *Nanoscale*, *8*(4), 2219-2226.

Li, H., He, X., Kang, Z., Huang, H., Liu, Y., Liu, J., ... & Lee, S. T. (2010). Water- soluble fluorescent carbon quantum dots and photocatalyst design. *Angewandte Chemie*, *122*(26), 4532-4536.

Li, H., Kang, Z., Liu, Y., & Lee, S. T. (2012). Carbon nanodots: synthesis, properties and applications. *Journal of materials chemistry*, *22*(46), 24230-24253.

Li, X., Shi, L., Li, L., Dong, C., Li, C. Z., & Shuang, S. (2019). Recent advances in carbon nanodots: properties and applications in cancer diagnosis and treatment. *Journal of Analysis and Testing*, *3*(1), 37-49.

Li, Z., Wang, L., Li, Y., Feng, Y., & Feng, W. (2019). Carbon-based functional nanomaterials: Preparation, properties and applications. *Composites Science and Technology*, *179*, 10-40.

Liu, C., Tang, B., Zhang, S., Zhou, M., Yang, M., Liu, Y., ... & Pang, D. W. (2018). Photoinduced electron transfer mediated by coordination between carboxyl on carbon nanodots and Cu2+ quenching photoluminescence. *The Journal of Physical Chemistry C*, *122*(6), 3662-3668.

Liu, D., Li, C., Zhou, F., Zhang, T., Zhang, H., Li, X., ... & Li, Y. (2015). Rapid synthesis of monodisperse Au nanospheres through a laser irradiation-induced shape conversion, self-assembly and their electromagnetic coupling SERS enhancement. *Scientific reports*, *5*(1), 1-9.

Liu, J., Li, D., Zhang, K., Yang, M., Sun, H., & Yang, B. (2018). One- step hydrothermal synthesis of nitrogen- doped conjugated carbonized polymer dots with 31% efficient red emission for in vivo imaging. *Small*, *14*(15), 1703919.

Liu, R., Li, H., Kong, W., Liu, J., Liu, Y., Tong, C., ... & Kang, Z. (2013). Ultra-sensitive and selective Hg2+ detection based on fluorescent carbon dots. *Materials Research Bulletin*, *48*(7), 2529-2534.

Liu, S., Tian, J., Wang, L., Zhang, Y., Qin, X., Luo, Y., ... & Sun, X. (2012). Hydrothermal treatment of grass: a low- cost, green route to nitrogen- doped, carbon- rich,

photoluminescent polymer nanodots as an effective fluorescent sensing platform for label- free detection of Cu (II) ions. *Advanced materials*, *24*(15), 2037-2041.

Liu, S., Yu, B., & Zhang, T. (2014). Nitrogen-doped carbon nanodots as a reducing agent to synthesize Ag nanoparticles for non-enzymatic hydrogen peroxide detection. *RSC Advances*, *4*(2), 544-548.

Liu, Y., Liu, Q., Wang, T., Bian, L., Zhang, S., Hu, H., ... & Jiang, Z. (2013). Circulating tumor cells in HER2-positive metastatic breast cancer patients: a valuable prognostic and predictive biomarker. *BMC cancer*, *13*(1), 1-8.

Lu, A. H., Salabas, E. E., & Schüth, F. (2007). Magnetic nanoparticles: synthesis, protection, functionalization, and application. *Angewandte Chemie International Edition*, *46*(8), 1222-1244.

Lv, Q., Gong, L., Zhang, T., Ye, J., Chai, L., Ni, C., & Mao, Y. (2016). Prognostic value of circulating tumor cells in metastatic breast cancer: a systemic review and meta-analysis. *Clinical and Translational Oncology*, *18*(3), 322-330.

Ma, Z., Ming, H., Huang, H., Liu, Y., & Kang, Z. (2012). One-step ultrasonic synthesis of fluorescent N-doped carbon dots from glucose and their visible-light sensitive photocatalytic ability. *New Journal of Chemistry*, *36*(4), 861-864.

Matea, C. T., Mocan, T., Tabaran, F., Pop, T., Mosteanu, O., Puia, C., ... & Mocan, L. (2017). Quantum dots in imaging, drug delivery and sensor applications. *International journal of nanomedicine*, *12*, 5421.

Meikle, T. G., Strachan, J. B., & Conn, C. E. (2019). Lipid nanomaterials for the delivery of antimicrobial peptides. *Methods in Microbiology*, *46*, 173-195.

Miao, P., Han, K., Tang, Y., Wang, B., Lin, T., & Cheng, W. (2015). Recent advances in carbon nanodots: synthesis, properties and biomedical applications. *Nanoscale*, *7*(5), 1586-1595.

Mihalache, I., Radoi, A., Mihaila, M., Munteanu, C., Marin, A., Danila, M., ... & Kusko, C. (2015). Charge and energy transfer interplay in hybrid sensitized solar cells mediated by graphene quantum dots. *Electrochimica Acta*, *153*, 306-315.

Mohammadi, S., & Salimi, A. (2018). Fluorometric determination of microRNA-155 in cancer cells based on carbon dots and MnO 2 nanosheets as a donor-acceptor pair. *Microchimica Acta*, *185*(8), 1-10.

Molaei, M. J. (2019). Carbon quantum dots and their biomedical and therapeutic applications: a review. *RSC advances*, *9*(12), 6460-6481.

Na, N., Liu, T., Xu, S., Zhang, Y., He, D., Huang, L., & Ouyang, J. (2013). Application of fluorescent carbon nanodots in fluorescence imaging of human serum proteins. *Journal of Materials Chemistry B*, *1*(6), 787-792.

Nawaz, F., Wang, L., Zhu, L. F., Meng, X. J., & Xiao, F. S. (2013). Ascorbic acid assisted green route for synthesis of water dispersible carbon dots. *Chemical Research in Chinese Universities*, *29*(3), 401-403.

Needham, D., Arslanagic, A., Glud, K., Hervella, P., Karimi, L., Høeilund-Carlsen, P. F., ... & Walke, P. (2016). Bottom up design of nanoparticles for anti-cancer diapeutics:"put the drug in the cancer's food". *Journal of drug targeting*, *24*(9), 836-856.

Nikalje, A. P. (2015). Nanotechnology and its applications in medicine. *Med chem*, *5*(2), 081-089.

Nikolova, M. P., & Chavali, M. S. (2020). Nanotechnology in medicine. In A. M. Gebauer, *Drug Discovery and Evaluation: Methods in Clinical Pharmacology* (pp. 533-546). Cham: Springer.

Ning, F., Shao, M., Xu, S., Fu, Y., Zhang, R., Wei, M., ... & Duan, X. (2016). TiO 2/graphene/NiFe-layered double hydroxide nanorod array photoanodes for efficient photoelectrochemical water splitting. *Energy & Environmental Science*, *9*(8), 2633-2643.

Ortega- Liebana, M. C., Encabo- Berzosa, M. M., Ruedas- Rama, M. J., & Hueso, J. L. (2017). Nitrogen- Induced Transformation of Vitamin C into Multifunctional Up-converting Carbon Nanodots in the Visible–NIR Range. *Chemistry–A European Journal*, *23*(13), 3067-3073.

Pantel, K., & Speicher, M. R. (2016). The biology of circulating tumor cells. *Oncogene*, *35*(10), 1216-1224.

Petcu, D., Zaharie, D., Gorgan, D., Pop, F., & Tudor, D. (2007, September). MedioGrid: a grid-based platform for satellite image processing. In *2007 4th IEEE Workshop on Intelligent Data Acquisition and Advanced Computing Systems: Technology and Applications* (pp. 137-142). IEEE.

Qu, S., Wang, X., Lu, Q., Liu, X., & Wang, L. (2012). A biocompatible fluorescent ink based on water- soluble luminescent carbon nanodots. *Angewandte Chemie international edition*, *51*(49), 12215-12218.

Qu, Z., Liu, P., Yang, X., Wang, F., Zhang, W., & Fei, C. (2016). Microstructure and characteristic of BiVO4 prepared under different pH values: photocatalytic efficiency and antibacterial activity. *Materials*, *9*(3), 129.

Reátegui, E., Aceto, N., Lim, E. J., Sullivan, J. P., Jensen, A. E., Zeinali, M., ... & Stott, S. L. (2015). Tunable nanostructured coating for the capture and selective release of viable circulating tumor cells. *Advanced materials*, *27*(9), 1593-1599.

Reiss, G., & Hütten, A. (2005). Applications beyond data storage. *Nature materials*, *4*(10), 725-726.

Rizzo, L. Y., Theek, B., Storm, G., Kiessling, F., & Lammers, T. (2013). Recent progress in nanomedicine: therapeutic, diagnostic and theranostic applications. *Current opinion in biotechnology*, *24*(6), 1159-1166.

Rogozea, E. A., Petcu, A. R., Olteanu, N. L., Lazar, C. A., Cadar, D., & Mihaly, M. (2017). Tandem adsorption-photodegradation activity induced by light on NiO-ZnO p–n couple modified silica nanomaterials. *Materials Science in Semiconductor Processing*, *57*, 1-11.

Ruiz, V., Yate, L., García, I., Cabanero, G., & Grande, H. J. (2017). Tuning the antioxidant activity of graphene quantum dots: Protective nanomaterials against dye decoloration. *Carbon*, *116*, 366-374.

Russell, A. W. (2013). Improving legitimacy in nanotechnology policy development through stakeholder and community engagement: forging new pathways. *Review of policy research*, *30*(5), 566-587.

Saha, R., Arunprasath, D., & Sekar, G. (2019). Surface enriched palladium on palladium-copper bimetallic nanoparticles as catalyst for polycyclic triazoles synthesis. *Journal of Catalysis*, *377*, 673-683.

Saleh, T. A., & Fadillah, G. (2019). Recent trends in the design of chemical sensors based on graphene–metal oxide nanocomposites for the analysis of toxic species and biomolecules. *TrAC Trends in Analytical Chemistry*, *120*, 115660.

Saxena, S. K., Nyodu, R., Kumar, S., & Maurya, V. K. (2020). Current advances in nanotechnology and medicine. In *NanoBioMedicine* (pp. 3-16). Springer, Singapore.

Sciortino, A., Cayuela, A., Soriano, M. L., Gelardi, F. M., Cannas, M., Valcárcel, M., & Messina, F. (2017). Different natures of surface electronic transitions of carbon nanoparticles. *Physical Chemistry Chemical Physics*, *19*(34), 22670-22677.

Sciortino, A., Cannizzo, A., & Messina, F. (2018). Carbon nanodots: a review—from the current understanding of the fundamental photophysics to the full control of the optical response. *C—Journal of Carbon Research*, *4*(4), 67.

Sciortino, A., Marino, E., Dam, B. V., Schall, P., Cannas, M., & Messina, F. (2016). Solvatochromism unravels the emission mechanism of carbon nanodots. *The journal of physical chemistry letters*, *7*(17), 3419-3423.

Shaw, J. A., Guttery, D. S., Hills, A., Fernandez-Garcia, D., Page, K., Rosales, B. M., ... & Coombes, R. C. (2017). Mutation analysis of cell-free DNA and single circulating tumor cells in metastatic breast cancer patients with high circulating tumor cell counts. *Clinical Cancer Research*, *23*(1), 88-96.

Shen, Z., Zhang, C., Yu, X., Li, J., Wang, Z., Zhang, Z., & Liu, B. (2018). Microwave-assisted synthesis of cyclen functional carbon dots to construct a ratiometric fluorescent probe for tetracycline detection. *Journal of Materials Chemistry C*, *6*(36), 9636-9641.

Shin, W. K., Cho, J., Kannan, A. G., Lee, Y. S., & Kim, D. W. (2016). Cross-linked composite gel polymer electrolyte using mesoporous methacrylate-functionalized SiO 2 nanoparticles for lithium-ion polymer batteries. *Scientific reports*, *6*(1), 1-10.

Shinde, D. B., & Pillai, V. K. (2012). Electrochemical preparation of luminescent graphene quantum dots from multiwalled carbon nanotubes. *Chemistry–A European Journal*, *18*(39), 12522-12528.

Simpson, A. M. (2015). Fabrication, Characterization, and Application of Carbon Nanoparticles for the Detection of Heavy Metal Ions in Aqueous Media.

Sivasankarapillai, V. S., Kirthi, A. V., Akksadha, M., Indu, S., Dharshini, U. D., Pushpamalar, J., & Karthik, L. (2020). Recent advancements in the applications of carbon nanodots: exploring the rising star of nanotechnology. *Nanoscale Advances*, *2*(5), 1760-1773.

Song, P., Zhang, L., Long, H., Meng, M., Liu, T., Yin, Y., & Xi, R. (2017). A multianalyte fluorescent carbon dots sensing system constructed based on specific recognition of Fe (III) ions. *RSC advances*, *7*(46), 28637-28646.

Sugiarti, S., & Darmawan, N. (2015). Synthesis of fluorescence carbon nanoparticles from ascorbic acid. *Indonesian Journal of Chemistry*, *15*(2), 141-145.

Sumi, N., & Chitra, K. C. (2019). Fullerene C60 nanomaterial induced oxidative imbalance in gonads of the freshwater fish, Anabas testudineus (Bloch, 1792). *Aquatic Toxicology*, *210*, 196-206.

Sun, Y. P., Zhou, B., Lin, Y., Wang, W., Fernando, K. S., Pathak, P., ... & Xie, S. Y. (2006). Quantum-sized carbon dots for bright and colorful photoluminescence. *Journal of the American Chemical Society*, *128*(24), 7756-7757.

Taniguchi, N. (1974). On the basic concept of nanotechnology. *Proceeding of the ICPE*.

Tao, H., Yang, K., Ma, Z., Wan, J., Zhang, Y., Kang, Z., & Liu, Z. (2012). In vivo NIR fluorescence imaging, biodistribution, and toxicology of photoluminescent carbon dots produced from carbon nanotubes and graphite. *Small*, *8*(2), 281-290.

Therese, G. H. A., & Kamath, P. V. (2000). Electrochemical synthesis of metal oxides and hydroxides. *Chemistry of materials*, *12*(5), 1195-1204.

Tiwari, J. N., Tiwari, R. N., & Kim, K. S. (2012). Zero-dimensional, one-dimensional, two-dimensional and three-dimensional nanostructured materials for advanced electrochemical energy devices. *Progress in Materials Science*, *57*(4), 724-803.

Umer, M., Vaidyanathan, R., Nguyen, N. T., & Shiddiky, M. J. (2018). Circulating tumor microemboli: Progress in molecular understanding and enrichment technologies. *Biotechnology advances*, *36*(4), 1367-1389.

Vijayakumar, R. A. (2013). Application of nanotechnology in medicine. In S. S. Navani NK, *Nanotechnology series. Volume 7: Diagnostics and therapeutics* (pp. 89-100). Houston: Studium Press LLC.

Wang, X., Feng, Y., Dong, P., & Huang, J. (2019). A mini review on carbon quantum dots: preparation, properties, and electrocatalytic application. *Frontiers in chemistry*, *7*, 671.

Wang, Y., & Xia, Y. (2004). Bottom-up and top-down approaches to the synthesis of monodispersed spherical colloids of low melting-point metals. *Nano letters*, *4*(10), 2047-2050.

Weiss, J., Takhistov, P., & McClements, D. J. (2006). Functional materials in food nanotechnology. *Journal of food science*, *71*(9), R107-R116.

Wu, W., He, Q., & Jiang, C. (2008). Magnetic iron oxide nanoparticles: synthesis and surface functionalization strategies. *Nanoscale research letters*, *3*(11), 397-415.

Xie, S., Su, H., Wei, W., Li, M., Tong, Y., & Mao, Z. (2014). Remarkable photoelectrochemical performance of carbon dots sensitized TiO 2 under visible light irradiation. *Journal of Materials Chemistry A*, *2*(39), 16365-16368.

Xu, J., Miao, Y., Zheng, J., Wang, H., Yang, Y., & Liu, X. (2018). Carbon dot-based white and yellow electroluminescent light emitting diodes with a record-breaking brightness. *Nanoscale*, *10*(23), 11211-11221.

Yan, Q. L., Gozin, M., Zhao, F. Q., Cohen, A., & Pang, S. P. (2016). Highly energetic compositions based on functionalized carbon nanomaterials. *Nanoscale*, *8*(9), 4799-4851.

Yang, Z., Li, Z., Xu, M., Ma, Y., Zhang, J., Su, Y., ... & Zhang, L. (2013). Controllable synthesis of fluorescent carbon dots and their detection application as nanoprobes. Nano-Micro Lett 5 (4): 247–259.

Yao, Y. Y., Gedda, G., Girma, W. M., Yen, C. L., Ling, Y. C., & Chang, J. Y. (2017). Magnetofluorescent carbon dots derived from crab shell for targeted dual-modality

bioimaging and drug delivery. *ACS applied materials & interfaces*, *9*(16), 13887-13899.

Yoo, H., Jung, H., Kim, S. A., & Mok, H. (2014). Multivalent comb-type aptamer–siRNA conjugates for efficient and selective intracellular delivery. *Chemical communications*, *50*(51), 6765-6767.

Yoon, H. J., Kim, T. H., Zhang, Z., Azizi, E., Pham, T. M., Paoletti, C., ... & Nagrath, S. (2013). Sensitive capture of circulating tumour cells by functionalized graphene oxide nanosheets. *Nature nanotechnology*, *8*(10), 735-741.

Zhang, H., Yee, D., & Wang, C. (2008). Quantum dots for cancer diagnosis and therapy: biological and clinical perspectives.

Zhang, J., Zhao, X., Xian, M., Dong, C., & Shuang, S. (2018). Folic acid-conjugated green luminescent carbon dots as a nanoprobe for identifying folate receptor-positive cancer cells. *Talanta*, *183*, 39-47.

Zhang, L., Ridgway, L. D., Wetzel, M. D., Ngo, J., Yin, W., Kumar, D., ... & Marchetti, D. (2013). The identification and characterization of breast cancer CTCs competent for brain metastasis. *Science translational medicine*, *5*(180), 180ra48-180ra48.

Zhang, M., Hu, L., Wang, H., Song, Y., Liu, Y., Li, H., ... & Kang, Z. (2018). One-step hydrothermal synthesis of chiral carbon dots and their effects on mung bean plant growth. *Nanoscale*, *10*(26), 12734-12742.

Zhang, M., Yuan, P., Zhou, N., Su, Y., Shao, M., & Chi, C. (2017). pH-Sensitive N-doped carbon dots–heparin and doxorubicin drug delivery system: preparation and anticancer research. *RSC advances*, *7*(15), 9347-9356.

Zhang, W., Wang, W., Yu, D. X., Xiao, Z., & He, Z. (2018). Application of nanodiagnostics and nanotherapy to CNS diseases. *Nanomedicine*, *13*(18), 2341-2371.

Zhang, W., Zeng, Z., & Wei, J. (2017). Electrochemical study of DPPH radical scavenging for evaluating the antioxidant capacity of carbon nanodots. *The Journal of Physical Chemistry C*, *121*(34), 18635-18642.

Zhang, X., Lai, Z., Liu, Z., Tan, C., Huang, Y., Li, B., ... & Zhang, H. (2015). A facile and universal top- down method for preparation of monodisperse transition- metal

dichalcogenide nanodots. *Angewandte Chemie International Edition*, *54*(18), 5425-5428.

Zhao, L., Geng, F., Di, F., Guo, L. H., Wan, B., Yang, Y., ... & Sun, G. (2014). Polyamine-functionalized carbon nanodots: a novel chemiluminescence probe for selective detection of iron (III) ions. *RSC Advances*, *4*(86), 45768-45771.

Zhong, Q., & Zhang, L. (2019). Nanoparticles fabricated from bulk solid lipids: Preparation, properties, and potential food applications. *Advances in colloid and interface science*, *273*, 102033.

Zhou, D., Li, D., Jing, P., Zhai, Y., Shen, D., Qu, S., & Rogach, A. L. (2017). Conquering aggregation-induced solid-state luminescence quenching of carbon dots through a carbon dots-triggered silica gelation process. *Chemistry of Materials*, *29*(4), 1779-1787.

Zhou, J., Deng, W., Wang, Y., Cao, X., Chen, J., Wang, Q., ... & Xu, X. (2016). Cationic carbon quantum dots derived from alginate for gene delivery: one-step synthesis and cellular uptake. *Acta biomaterialia*, *42*, 209-219.

Zhou, Y., Dong, C. K., Han, L. L., Yang, J., & Du, X. W. (2016). Top-down preparation of active cobalt oxide catalyst. *ACS Catalysis*, *6*(10), 6699-6703.

Zhu, A., Luo, Z., Ding, C., Li, B., Zhou, S., Wang, R., & Tian, Y. (2014). A two-photon “turn-on” fluorescent probe based on carbon nanodots for imaging and selective biosensing of hydrogen sulfide in live cells and tissues. *Analyst*, *139*(8), 1945-1952.

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