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Synthesis and Applications of Carbon Dots

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UNIVERSITY OF MIAMI

SYNTHESIS AND APPLICATIONS OF CARBON DOTS

By

Yiwen Ji

A DISSERTATION

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the requirements for the degree of
Doctor of Philosophy

SYNTHESIS AND APPLICATIONS OF CARBON DOTS

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Carbon dots (C-Dots) are recognized as excellent, zero-dimensional, bio-compatible nanoparticles with diameters less than 10 nm. Since its discovery in 2004 by unintentional single-wall nanotube purification, C-Dots have been extensively studied and applied in many aspects in the past couple decades. C-Dots are mainly made from carbon source and nitrogen/sulfur doping, which made them bio-compatible and non-toxic nanomaterials, in comparison to other nanoparticles, such as semi-conductor quantum dots. In addition to non-toxicity, C-Dots possess excellent photostability and high fluorescence (QY up to 94 %), which is comparable to quantum dots for bio-imaging applications. In contrast to quantum dots, the synthesis of C-Dots is usually simple and inexpensive. This lowers the cost of commercializing C-Dots as biomarkers, metal sensors, optical sensing, light harvesting, fluorescent ink, anti-counterfeiting, and potential drug delivery system for example.

To further gain fundamental understanding and for better utilization of C-Dots’ optical properties, the molecular origin of C-Dots’ photoluminescence properties is indispensable. Studies revealed that C-Dots can be formed at high temperature and high pressure and have similar photophysical behavior in spite of having different starting materials. It would be beneficial to look further into both the physical and optical properties of C-Dots.
In terms of physical properties of C-Dots, their sizes and shapes are well-studied. However, C-Dots synthesized from citric acid and ethylenediamine at particular experimental conditions, have a unique physical gel-like property. Herein, the physical (viscosity) properties are examined in depth to understand its gel-like characteristics.

Besides the special physical properties, the issue of C-Dots favoring fluorescence remains debatable. To obtain a further understanding of the C-Dot photoluminescence mechanism, two series of synthetic building blocks are proposed and tested.

A series of carbon-source building blocks were selected to study the influence of varying numbers of carbon atoms or carboxyl/hydroxyl groups on the fluorescence mechanism of C-Dots. The conclusion provides insights for future research when certain photophysical properties are desired.

C-Dots are often synthesized using a carbon source as well as a doping agent, e.g. nitrogen. In addition to carbon-source building blocks, another series of nitrogen-source building blocks were chosen to understand further the synthetic mechanism. Fluorescent amino acid, namely tryptophan, and two different nitrogen dopants, ethylenediamine and urea, were chosen as nitrogen-source building blocks to investigate the C-Dot photoluminescence mechanism in detail.
To My Advisor
ACKNOWLEDGEMENTS

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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>2D</td>
<td>two dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>three dimensional</td>
</tr>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>ATR-FTIR</td>
<td>attenuated total reflection Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
</tr>
<tr>
<td>BDA</td>
<td>1,4-butanediamine</td>
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<td>BSA</td>
<td>bovine serum albumin</td>
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<td>C-Dots</td>
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<td>citric acid</td>
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<td>CNT</td>
<td>carbon nanotubes</td>
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<td>DLS</td>
<td>dynamic light scattering</td>
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<td>ECL</td>
<td>electrochemiluminescence</td>
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<td>ethylenediamine</td>
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<tr>
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<td>electrospray ionization mass spectrometry</td>
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<td>FA</td>
<td>formic acid</td>
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<td>Fourier transform infrared spectroscopy</td>
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<td>GA</td>
<td>glutamic acid</td>
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<tr>
<td>HMTA</td>
<td>hexamethylene tetramine</td>
</tr>
<tr>
<td>HR-TEM</td>
<td>high resolution transmission electron microscopy</td>
</tr>
<tr>
<td>AA</td>
<td>aspartic acid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
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<tr>
<td>LVR</td>
<td>linear viscoelastic region</td>
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<td>molecular weight cut-off</td>
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<td>near-infrared</td>
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Chapter 1 Introduction

1.1 Brief History of Carbon Nanomaterials

Carbon materials are widely used in nanoscale material science: from zero (e.g. carbon dots, fullerene), one (e.g. single-walled nanotube), to two dimensional nanomaterials (such as graphene, graphene oxide). During the past two decades, carbon was used as a benign substitution candidate for highly fluorescence quantum dots. Quantum dots often have a heavy metal core, therefore has intrinsic toxicity and tedious preparation steps. The new candidate is called carbon dots (C-Dots), which is named after its main compositional element.

Because of its biocompatible composition and straightforward synthesis, C-Dots have become the center of numerous research efforts. Later, C-Dots were discovered to have favorable photophysical properties that are quantitatively comparable to semiconductor-based quantum dots. C-Dots have superior optical properties, such as two-photon imaging, excitation-wavelength-dependent photoluminescence emission, size dependent photoluminescence emission, and photobleaching resistance. Some of these properties are unique for C-Dots, but not for quantum dots.

In addition to its advantageous optical properties, C-Dots is bio-friendly to cells and has capability for various surface modifications. These beneficial physical characters facilitate many applications of C-Dots, such as medical bone imaging, targeted drug delivery, etc.
1.2 Categories

C-Dots was first discovered in 2004 by “accident” via arc-discharging single-walled carbon nanotubes (SWCNTs). Some fluorescent bands were isolated after gel-electrophoresis. These bands have relatively spherical shapes, and later named carbon dots (C-Dots). Arc-discharge is a harsh experimental condition. Various preparation methods were tested to simplify the synthesis. These C-Dots synthetic routes can be categorized into two groups: top-down and bottom-up.

Top-down groups contain the C-Dots synthesized from relatively large carbon source molecules. Those big carbon molecules were then gradually broken down into smaller carbon atoms and formed the C-Dots carbogenic core. Some large carbon source molecules are: carbon nanotubes, carbon soot, graphite electrode rod, etc. Since C-Dots were first discovered using arc-discharge method. Researchers followed the same patterns to break down large carbon precursors. Breaking down the carbon sources could be difficult; thus, the experimental conditions are usually harsh, such as using arc-discharge, electrochemical etching, and laser ablation. Examples will be given for each top-down route in Chapter 3. In general, synthetic methods became more, and more mild.

The bottom-up category developed around 2008 to replace harsh and dangerous top-down methods. Synthesis of C-Dots then became less expensive and easier to reproduce in almost all chemistry laboratories. Bottom-up category includes all the other methods that build C-Dots from a (relatively) small carbon precursor and some doping materials. Various carbon precursors and dopants were tested. Some commonly used carbon precursors include, but not limited to, citric acid, citrate salt, and sugar. All of them are affordable and readily available in chemistry labs. There are undeniable
advantages for using affordable starting materials and mild synthetic methods over expensive chemicals (SWCNTs cost on average $600 to $1000 for 50g), and require harsh conditions. Compared to top-down methods, bottom-up methods use more common starting materials and has a much less harsh approach. The experimental conditions are often mild, which mainly involve heating (200–300°C) and drying. In certain cases, the syntheses were one-pot syntheses. Thermal pyrolysis, microwave, and ultrasound methods fell into the bottom-up category and serve the purpose well. Some representative examples are given later in Chapter 3.

Both synthetic routes produce C-Dots with similar photophysical properties and physical sizes, below 10 nm. Bottom-up methods became the majority method since 2008 and completely replaced top-down methods by 2010. After the bottom-up approach became dominant, more and more research have been carried out on C-Dots. Photoluminescence and the (bio) applications are still hot topics.

Different from its predecessor, quantum dots, or other fluorophores, the fluorescence emissions of (most) C-Dots are dependent on excitation wavelength. There are some hypotheses on why C-Dots have such excitation-wavelength-dependent emission behaviors. Some literatures suggested that the photoluminescence of C-Dots are due to the free zigzag edge. Some have proposed synergistic effect of both molecular state and core state of C-Dots. Others have shown its presence via fluorescent by-products. The excitation-wavelength-dependent emissions probably result from a mixture of different effects mentioned before. These hypotheses are introduced in Chapter 3. Nonetheless, the fluorescence mechanism of C-Dots still remains inconclusive, and there is no clear consensus was reached by the academia.
1.3 Applications

Research on C-Dots are mostly focused on synthesis and characterization since its discovery. Plenty of synthesis methods are reported, and hypotheses on the origin of photoluminescence are proposed. Besides studying the synthesis and mechanism and exploring more synthetic methods, the applications of C-Dots are important topics as well. Practical applications can drive the research of C-Dots further and longer.

Since C-Dots have many hydrophilic surface functional groups, e.g., hydroxyl groups and carboxyl groups, C-Dots have great potential for surface modification, especially when C-Dots also have large surface to volume ratio. The surface functionalizations of C-Dots and their applications have become the center of C-Dot related researches since 2013. To name a few applications for C-Dots: energy conversion/storage, bio-imaging, drug delivery, metal sensors, diagnostics devices, and multicolor inks.

More insights and experiments are expected. To commercialize these C-Dots applications for daily life or in the scientific lab, it will take tremendous effort and time. This would be the next focus point for C-Dots in the next decade.

1.4 Summary

C-Dots gained much attention since its appearance in 2004, which required harsh conditions and expensive chemical materials. Between 2004 and 2008, more synthetic methods have been explored, and since 2008, bottom-up methods became dominant because of the mild experimental conditions and affordable starting materials.
C-Dots have distinctive photoluminescence quantum yield and very unique optical properties, its emission wavelength often undergo a red shift as the excitation wavelength red-shifted. To further understand the behavior, three hypotheses were proposed by researchers. However, no consensus has been reached.

Despite numerous efforts that have been made on finding applications of C-Dots, more research and analysis are needed in order to further use and commercialize C-Dots.
Chapter 2 Methodology

2.1 Materials

Formic acid (88 %) was purchased from J.T.Baker (Center Valley, PA). Ethylenediamine was obtained from MP Biomedical (Santa Ana, CA). Oxalic acid and citric acid were from VWR (Radnor, PA). L-aspartic acid and L-glutamic acid were purchased from Sigma Aldrich (St. Louis, MO). Ethylenediamine was purified using simple distillation (120°C boiling point temperature in a sand bath) before use and stored with a trace amount of sodium hydroxide to avoid oxidation. Quinine hemisulfate monohydrate (99 %) was purchased from Alfa Aesar (Ward Hill, MA), and N, N’-dimethyl-9,9’-biacridinium dinitrate lucigenin was purchased from AdipoGen Life Science (San Diego, CA). All chemicals were used without any further purification unless otherwise specified. Ultrapure water was obtained from an Elga PURELAB Ultra water purifier (Woodridge, IL) with a resistivity of 18 MΩ cm, surface tension of 72.6 mN m⁻¹, and pH 5.6 at 20.0 ± 0.5°C. Dialysis was run through a cellulose ester membrane with molecular weight cut-off of 100-500 Daltons purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA).

2.2 Synthesis of C-Dots

In general, 1 molar equivalence of the selected acid was added into 14 molar equivalents of the nitrogen dopant, i.e. ethylenediamine, in round bottom flask and reacted at 180 °C for 4 h in an oil bath under argon to avoid the oxidation of ethylenediamine. The products were then evaporated using a rotovap for 3 h to eliminate
the excessive ethylenediamine, and underwent prolonged dialysis (molecular weight cutoff 100-500 Da) for five days. The carbon dots dispersed in water, and characterized.

In Chapter 5, since a new starting material, i.e. urea, was used and it is a solid, the purification process was slightly changed. To remove excess starting material from the reaction pot, a gel electrophoresis was carried using a 10 % agarose gel with a 0.1 M Bis-Tris buffer (pH = 5.9).

2.3 Instrumentation

2.3.1 Spectroscopy

Spectroscopy is very informative when studying molecular surface structures and optical properties. To study the effects of different building blocks (i.e. different acids) on the synthesis of carbon dots, we used the following spectroscopic techniques: ultraviolet–visible, fluorescence, attenuated total reflection Fourier transform infrared (ATR-FTIR), and X-ray photoelectron spectroscopies. Zeta potential measurements were also utilized to characterize the C-Dots.

2.3.1.1. UV-visible Spectroscopy

Absorption spectra were performed on an Agilent Technologies Cary 100 UV-Vis spectrophotometer and a 10 mm path length UV cell from Starna Cells (Atascadero, CA). The spectrum covered the region from 200 to 800 nm.
2.3.1.2. Fluorescence Spectroscopy

Photoluminescence data were carried out using a Horiba Jobin Yvon Fluorolog-3 (Kyoto, Japan) with a slit width of 5 nm for both excitation and emission and a 10 mm path length fluorescence Starna cell (9F-Q-10). The emission spectrum covered the range from 325 to 625 nm. The quantum yield was calculated via the luminescence measurement, using quinine sulfate as a reference standard, with the following:

\[ \Phi = \Phi_R \times \frac{I_R}{I} \times \frac{A_R}{A} \times \frac{\eta^2}{\eta_R^2} \]

where \( \Phi \) is the quantum yield, \( I \) is the measured integrated emission intensity, \( \eta \) is the refractive index of the solvent, \( A \) is the optical density, and the subscript \( R \) refers to the reference standard.

2.3.1.3. Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy

The attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra were measured on a Perkin-Elmer Frontier using ATR technique with air as reference. The ATR-FTIR spectra covered region from 500 to 4000 cm\(^{-1}\).

2.3.1.4. X-ray Photoelectron Spectroscopy (XPS)

X-ray photoelectron spectroscopic spectra (XPS) were obtained using a Perkin-Elmer PHI 560 ESCA system with a double-pass cylindrical mirror analyzer operated at 225 W and 12.5 kV using a Mg K\( \alpha \) anode and a \( h\nu = 1253.6 \) eV photon energy. Core levels of the C 1s and O 1s orbitals were scanned and intensities were normalized according to their respective atomic sensitivity factors.
2.3.1.5. Zeta Potential and Dynamic Light Scattering (DLS)

Zeta potential measurements were recorded using a Zetasizer Nano-ZS from Malvern Instruments (Westborough, MA) with a standard 633 nm laser. Sample solutions were aliquoted into disposable folded capillary cells (DTS1070) from Malvern Instruments (optical path length 5 mm). The instrument is also capable of measuring dynamic light scattering of biomaterials, such as protein and oligonucleotides. However, in the case of our C-Dots, no consistent size information was obtained from the Zetasizer.

2.3.2 Microscopy

Microscopy is a powerful tool for revealing 2D and 3D structures of the samples. To visualize the structures of synthesized C-Dots, samples were scanned with atomic force microscopy (AFM) and transmission electron microscopy (TEM).

2.3.2.1. Atomic Force Microscope (AFM)

Atomic force microscope (AFM) images were carried out using Agilent 5420 AFM to measure the thickness of samples (Z-axis). The AFM was installed in a clean room (class 1000) with constant temperature of 20.0 ± 0.5 °C and humidity of 50 ± 1 %. Tapping mode was used with cantilever resonance frequency around 160 kHz and force constant 21 N/m. Tapping mode cantilever (PPP-NCL-10) was purchased from NanoSensors (Neuchâtel, Switzerland). Samples were diluted in aqueous solution and drop-casted onto a freshly cleaved mica surface. After air-drying for 2 hours in a clean room, the aliquot samples were scanned by AFM using tapping mode. Extracted profiles were reproduced several times to ensure that the results reflected the sample as a whole.
2.3.2.2. Transmission Electron Microscope (TEM)

High-resolution transmission electron microscope (HR-TEM) images were taken using a FEI Tecnai F30 TEM (300 kV) in Advanced Materials Processing and Analysis Center at the University of Central Florida (Orlando, FL). Aqueous samples were then drop-casted onto a carbon grid and let air-dried for at least 2 hours before scan.

2.3.3 Rheology

Our C-Dots samples have a gel-like phase when first synthesized from starting materials. To study the gel-likeness further, the rheological properties of the carbon dot material were investigated by Anton Paar MCR-102 Rheometer equipped with a Peltier system of temperature control with an accuracy of ± 0.01 °C in Ahmad Lab at University of Kashmir (J&K, India). The rheological measurements were performed using CP25-2 (diameter = 25 cm, cone angle = 1.998˚) measuring system. Test modes employed were namely: the shear rate sweep, structure decomposition and regeneration (thixotropy) test, and frequency sweep. In steady shear experiments, shear rate was varied from 0.1 to 10000.0 sec⁻¹. To control shear rate (CSR) profile for obtaining the thixotropic behavior, time dependent viscosities in three shear intervals viz low, high and again low shear in steps were plotted as a function of time. Stress sweep was performed on the carbon dot material at a constant frequency of 10 rad•sec⁻¹ to determine the linear viscoelastic region (LVR). The material was also subjected to a frequency sweep in LVR (stress of 0.1 Pa) to study the viscoelastic performance over a wide range of angular frequencies ranging from 0.01 to 600.00 rad•sec⁻¹. All experiments were performed in triplicate to ensure reproducibility.
Chapter 3 Synthesis of C-Dots: Generality

Being a great non-toxic, low cost, highly fluorescent nanomaterial, carbon dots (C-Dots) have drawn tremendous attention in the past two decades. Compared to its predecessor, quantum dots, it has advantages of biocompatibility, high throughput, and equal photoluminescence ability. To have a better insight of C-Dots, a brief history of C-Dots synthesis methods will be discussed in this chapter.

Generally, C-Dots are divided into two categories: top-down and bottom-up based on synthetic approaches.\(^\text{10}\)

Top-down methods are physical approaches, which require external energy to break down relatively large carbon molecules into smaller carbogenic parts. These small carbon elements that were broken off from large molecules later lay a foundation for C-Dots. The first top-down method is arc-discharge where candle soot was arc-discharged to produce single-walled carbon nanotubes (SWCNT) and accidentally found C-Dots formation while purifying. After 2004, more physical approaches were explored, such as laser ablation,\(^\text{11}\) electrochemical etching,\(^\text{12}\) electro-oxidation,\(^\text{13}\) etc.

On the other hand, bottom-up methods are chemical approaches, which use carbon precursors to synthesize C-Dots. Bottom-up methods appeared four years later after the first discovery of C-Dots. The reason is obvious. Bottom-up methods use lower cost starting materials and experimental apparatus, and the experimental ratio and conditions are easier to control than top-down methods. Beginning in 2008, more carbon precursors have been explored: citric salts, citric acids, urea, glucose, or even milk\(^\text{14}\) and orange juice.\(^\text{15}\) Most synthetic routes need oxidation treatment and purification process after reactions. More detail will be discussed below.
3.1 Top-Down Methods

3.1.1 History and Discovery

C-Dots were first discovered using a top-down method in 2004.\textsuperscript{3} Candle soot was arc-discharged to produce single-walled carbon nanotubes (SWCNTs), and then was oxidized with nitric acid to improve hydrophilicity. SWCNTs were then extracted with basic solution. The black suspension was purified using gel-electrophoresis and resulted in two black bands (long and short tubular SWCNTs) and one fast moving fluorescent band. The fluorescent band was later isolated using nominal molecular weight cut off (MWCO) Centricon filtration devices into three samples, and they emitted green-blue, yellow, and orange fluorescence upon excitation at 365 nm in order of their elution and increasing sizes. These fluorescence carbon based materials are later named C-Dots due to their sizes (lateral size: 18 nm and height around 1 nm by AFM). Quantum yield of the yellow fluorescent segment was measured to be 0.016 (quinine sulfate as reference). FTIR suggested the presence of carboxyl functional groups on the surface of C-Dots. Furthermore, FTIR showed no C-H out-of-plane bending, which means the fluorescence was not originated from polyaromatic hydrocarbons. Energy disperse X-ray spectroscopy elemental analysis indicated that the surface layers of C-Dots consisted of 53.93 % C, 2.56 % H, 1.20 % N, and 40.33 % O.

Although the discovery of C-Dots was unintentional, the topic of C-Dots has gained marvelous attention from researchers. Arc-discharge is not a readily available experiment apparatus for many researchers, therefore different physical approaches (Figure 3.1), such as laser ablation,\textsuperscript{11} electrochemical etching,\textsuperscript{12,16} and electrochemical oxidization,\textsuperscript{13} were explored to optimize and ease the C-Dots synthesis. These top-down
physical approaches break down of larger carbon source, like candle soot,\textsuperscript{3} graphite powder and cement\textsuperscript{11} or even graphene,\textsuperscript{12} and C-Dots produced from these materials have excitation wavelength dependency. Details are summarized in the figure below.

\textbf{Top-Down Method Timeline}

\begin{itemize}
  \item \textbf{Arc-discharge}
    \begin{itemize}
      \item Material: candle soot
      \item Scrivens et al. (Univ of S. Carolina)
      \item JACS 2004, \textit{126}, 12736-12737
    \end{itemize}
  \item \textbf{Laser ablation}
    \begin{itemize}
      \item Material: graphite powder + cement
      \item Sun et al. (Clemson Univ)
      \item JACS 2006, \textit{128}, 7756-7757
    \end{itemize}
  \item \textbf{Electro-oxidation}
    \begin{itemize}
      \item Material: Graphite
      \item Pang et al. (Wuhan Univ, CN)
      \item Chem. Comm. 2008, 5116-5118
    \end{itemize}
  \item \textbf{Arc-produced}
    \begin{itemize}
      \item Material: carbon black, graphite, carbon nanotubes (CNT)
      \item Mustelin et al. (Burnham Inst. for Med. Res)
    \end{itemize}
  \item \textbf{Electrochemical etching}
    \begin{itemize}
      \item Material: graphene + CNT
      \item Ding et al. (Univ of Western Ontario, CA)
      \item JACS 2007, \textit{129}, 744-745
    \end{itemize}
  \item \textbf{Electrochemical}
    \begin{itemize}
      \item Material: graphite rod
      \item Chi et al. (Fuzhou Univ, CN)
      \item JACS 2009, \textit{131}, 4564-4565
    \end{itemize}
\end{itemize}

\begin{figure}[h]
  \centering
  \includegraphics[width=\textwidth]{figure31.png}
  \caption{Timeline of top-down synthetic approaches of C-Dots}
  \label{fig:figure31}
\end{figure}

\subsection{3.1.2 Top-Down Synthetic Routes}

Arc-discharge methods were used by Scrivens et al. in 2004,\textsuperscript{3} followed by Mustelin et al. in 2006.\textsuperscript{17} Mustelin and co-workers purchased arc-produced pristine and oxidized carbon nanotubes (CNTs) and separated them using nominal molecular weight cutoff (MWCO) membranes (<3, 3-10, 10-30, and 30-100 kDa). Both CNTs, from two different commercial sources (Carbon Solutions, Inc., Riverside, CA and Nanocs, Inc., New York, NY), produced fluorescent nanoparticles after ultracentrifugation. Pristine CNTs (pCNTs) produced a strong blue fluorescent segment after cup-horn sonication and ultracentrifugation through MWCO membranes of 10-30 kDa. Oxidized CNTs (oxCNTs)
had size-dependent fluorescence behavior, generating greenish-blue to yellowish-green with increasing molecular sizes. It was shown that both pCNTs and oxCNTs had fluorescent C-Dots after purification regardless of different suppliers.

Electric arc-discharge method is a relatively harsh experimental condition, therefore researchers started to explore other physical approaches that are more available in conventional laboratories, such as laser ablation,\textsuperscript{11} and electrochemical exfoliation.\textsuperscript{12,13,16} These preparations usually involve strong acid oxidation and purification processes.

Other than purchasing arc-produced CNTs and purify for C-Dots, C-Dots have been produced intentionally by Sun\textsuperscript{11} and co-workers. Using laser ablation, carbon elements are broken off from the mixture of graphite powder and cement themselves in the presence of H\textsubscript{2}O vapor with argon as carrier gas. The as-obtained carbon particles were in various sizes and no photoluminescence present. The sample was later treated with 2.6 M HNO\textsubscript{3} solution for 12 h and surface passivated with polymers, such as PEG\textsubscript{1500N} (diamine-terminated oligomeric poly(ethylene glycol) H\textsubscript{2}NCH\textsubscript{2}(CH\textsubscript{2}CH\textsubscript{2}O)\textsubscript{n}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}NH\textsubscript{2}, average n \textasciitilde 35) or poly(propionylethyleneimine-co-ethyleneimine) (PPEI-EI), at 120°C for 72 h. The sample was then dispersed in water and centrifuged for 30 min for purification. The PEG\textsubscript{1500N} passivated C-Dots emitted bright excitation wavelength dependent color (Figure 3.2) and outstanding photo-bleaching resistance. The size of these C-Dots (5 nm in diameter) was relatively uniform and confirmed by AFM. These C-Dots are also excitation wavelength dependent, which later became a characteristic feature to identify C-Dots. The PL quantum yield (QY) can
reach up to 0.20 at 400 nm excitation. The C-Dots with more polymer surface passivation on the surface had improved QY.

![Figure 3.2](image)

Figure 3.2 PEG<sub>1500N</sub> passivated C-Dots emitted different color when excited at different wavelength (excitation wavelengths shown on the figure). Bare C-Dots did not emit any fluorescence without acid-treatment and surface passivation.

Other than laser ablation, electrochemical method is another preparation method. In 2007, Ding et al.<sup>12</sup> have found that the electrolyte solution, from oxidizing multiwalled carbon nanotubes (MWCNTs) covered working electrodes in acetonitrile solution with a Pt wire counter electrode and an Ag/AgClO<sub>4</sub> reference electrode in 0.1 M tetrabutylammonium perchlorate (TBAP), contained blue fluorescent C-Dots. After evaporating the acetonitrile, the C-Dots were isolated and dispersed in aqueous solution
for dialysis using a cellulose ester membrane bag. The C-Dots absorbed at 270 nm and emitted at 410 nm, and the emission peaks were excitation wavelength dependent (Figure 3.3). HR-TEM data suggested that the C-Dots have a relatively uniform size, $2.8 \pm 0.5$ nm and a lattice spacing distance of 3.1 to 3.4 Å. Raman spectra indicated that $sp^2$ and disordered $sp^3$ carbon were both present. The authors therefore hypothesized that the excitation dependent emission was due to variable emission sites and surface states.

![Figure 3.3](image)

**Figure 3.3** Excitation wavelength dependent fluorescence emission of C-Dots.

Instead of covering the working electrode with starting materials via chemical vapor deposition, Pang and co-workers\textsuperscript{13} succeed in obtaining C-Dots by electro-oxidizing a bare graphite column electrode with a Pt wire counter electrode in 0.1 M
NaH$_2$PO$_4$ aqueous solution. The dark brown solution was centrifuged (28,000g) for 30 min and ultrafiltered through <5 and 5-10 kDa molecular weight cutoff membranes. The C-Dots filtered were then examined by high-resolution transmission electron microscope (HRTEM) which revealed that C-Dots were monodispersed and had graphite-like lattice spacing of 3 Å (Figure 3.4).

The smaller sized C-Dots passed through <5 kDa membranes were around 2 nm in diameter as measured by HRTEM and had quantum yield of 0.012; while the larger
size C-Dots (5-10 kDa) were about 3 nm in diameter. The smaller and larger C-Dots have different fluorescence colors, blue and yellow, respectively, which indicates that the emission peak is size dependent (Figure 3.5).

![Figure 3.5 Size dependent emission spectra of < 5 kDa C-Dots (left) and 5-10 kDa C-Dots (right).](image)

Both C-Dots, however, have emission spectra that were independent on excitation wavelength, unlike previous reported C-Dots. These C-Dots were stable in solution, up to 2 M KCl (pH = 7.0), and responsive to ambient pH environment (highest fluorescence at pH = 4.5).

Furthermore, electrochemically synthesized C-Dots can be used to generate electrochemiluminescence (ECL) signal. Nanosized (2 nm in diameter), monodispersed, water-soluble C-Dots were obtained by electro-oxidizing graphite working electrode in phosphate buffer solution with a Pt mesh counter electrode and a Ag/AgCl reference electrode. The study shown that C-Dots’ ECL behavior was very similar to those of
semiconductor nanocrystals, therefore the authors proposed that the ECL emission mechanism was due to the excited-state C-Dots ($R^\ast$) via electron-transfer annihilation of negatively charged ($R^{\ast\ast}$) and positively charged C-Dots ($R^{\ast\ast\ast}$) (Figure 3.6). Since C-Dots had a higher ECL emission peak wavelength (535 nm) than PL emission peak (455 nm), it was concluded that the energy gap of C-Dots surface states (for ECL emission) was smaller than the band gaps of C-Dots core (for PL).

Figure 3.6 Scheme showing the ECL and PL mechanism hypothesis of C-Dots. $R^{\ast\ast\ast}$, $R^{\ast\ast}$, and $R^\ast$ represents negatively charged, positively charged and excited state of C-Dots, respectively.

These approaches above are often costly, require specific expensive instruments, sometimes it could be hard to control the size uniformity, and requires post-synthesis functionalization for C-Dots dispersibility. In the light of having a more facile synthesis method and more systematic outcomes, bottom-up syntheses became dominant after 2008-2009.
3.2 Bottom up Methods

3.2.1 History

Despite the fact that C-Dots were discovered using top-down methods, bottom-up synthesis routes became wildly pursued to better control product uniformity, simplify functionalization, and lower cost. Unlike top-down synthesis, bottom-up synthesized C-Dots are often solely based on its chemistry, and utilize carbon molecular precursors, which later form the part of the infrastructure/core structure of C-Dots. The sample dispensabilities are improved significantly, both in aqueous solution and organic solvents. Tremendous carbon-based starting materials have been explored: from the materials used before in top-bottom, like candle soot,\textsuperscript{18} gas burner soot,\textsuperscript{19} to more materials, such as graphite rods,\textsuperscript{20} ammonium citrate salt,\textsuperscript{21} phenol/formaldehyde resin,\textsuperscript{22} polyethylene glycol (PEG),\textsuperscript{23} etc. Most of bottom-up synthesis use relatively mild experimental conditions, compared to top-down methods. A few representative examples are given below.

![Timeline of bottom-up synthetic approaches of C-Dots](image)

Figure 3.7 Timeline of bottom-up synthetic approaches of C-Dots
3.2.2 Bottom-up Synthetic Routes

Bottom-up synthetic routes are very diversified (Figure 3.7): applying oxidation, heat (combustion or thermal), microwave, etc. Some representative examples are given below.

Hydrothermal-pyrolysis synthesis of C-Dots was soon being examined by Giannelis et al., and they were able to obtain monodisperse and size < 10 nm C-Dots using either citrate salts or 4-aminoantipyrine as precursors. Water soluble C-Dots can only be synthesized from citrate salts precursors with citric acid, not from 4-aminoantipyrine. Pyrolysis of citric acid alone resulted in insoluble carbogenic solid only, thus citrate salts played an important role in fabricating C-Dots. The authors proposed that the amide linkage (-NHCO-), from 2-(2-aminoethoxy)-ethanol (HOCH₂CH₂OCH₂CH₂NH₃⁺) citrate salt, tethered the organic corona covalently to the carbogenic cores from citrate units carbonization to form C-Dots. The carbogenic core had a similar stoichiometry with graphite oxide according to elemental analysis. Quantum yield of this water dispersed C-Dots is 3 %. However, the C-Dots had PL emissions that were independent of excitation wavelength, unlike most previously reported C-Dots.

Besides the most common synthesis method, hydrothermal synthesis, other synthetic routes were investigated, such as ultrasound techniques. In 2010, Kang et al., for the first time, synthesized C-Dots monodisperse water-soluble C-Dots from glucose by acid or base assisted ultrasonic treatment for 4 h. The C-Dots product powders were collected in two ways: one way is to acid treat and then oven-dried at 80°C for 6 h; and the other way is to base treat (neutralized with HCl), and then dissolved in ethanol under stirring before removing salts using MgSO₄ and air-dried. The sizes of these C-Dots were
around 5 nm and they had the characteristic excitation wavelength dependent emissions (Figure 3.8), which confirmed C-Dots were successfully synthesized using ultrasonic methods.

Figure 3.8 (a) TEM image of C-Dots with diameter less than 5 nm; (b) C-Dots in water; (c) C-Dots at 365 nm excitation; (d) (e) (f) (g) fluorescent microscopic images of C-Dots under 360, 390, 470, and 540 nm excitation, showing the excitation wavelength dependent emission behavior.

More interestingly, these C-dots had PL emissions that extended into the NIR wavelength range, and they could be excited by NIR wavelengths (700 – 1000 nm) and emitted in shorter wavelength range from 450 to 750 nm (Figure 3.9). This property made these C-Dots a powerful energy transfer candidate in biology imaging, where noninvasive and deep penetration of NIR radiation are in high demand.
Figure 3.9 Up-conversion photoluminescent spectrum showing C-Dots can absorb NIR excitation and emit at shorter wavelength.

After intense research, the starting materials have become more and more concise. Giannelis et al.\textsuperscript{25} have followed up on bottom-up synthetic route to investigate systematically the formation mechanism of C-Dots. Citric acid and ethanolamine were chosen as starting materials. C-Dots were then synthesized using pyrolysis method. Before, excitation wavelength dependency in emission spectra was considered a characteristic feature of C-Dots, along with the TEM/AFM confirmed nanosize; however, later in many literatures, using both top-down and bottom-up synthetic routes, suggested some C-Dots could have emission spectra that were excitation independent as well. In 2012, Giannelis et al. proposed a hypothesis that the two kinds of emission behaviors were due to a mixture of two different products.\textsuperscript{25} Temperature, in this case 180, 230, and 300°C, played a significant role in determining which product would be dominant in the synthesis. Citric acid monohydrate and ethanolamine were mixed in a molar ratio of 1:3 and treated at 180°C for 30 min under reflux in air, before purifying via 3.5 kDa dialysis membrane. Highly fluorescent (QY~ 0.50, anthracene as reference), excitation
wavelength independent emission, bright yellow solution was obtained; nonetheless, no nanoparticles were observed by TEM or DLS. Scheme 1 was confirmed by FTIR, XPS, $^1$H and $^{13}$C NMR, and ESI-MS. The PL model of this amide is a direct singlet-to-triplet transition.

Scheme 1 Reaction scheme of citric acid and ethanolamine at 180°C

To obtain real spherical C-Dots, the pyrolysis needed a higher temperature, 230°C. The product solution would be a mixture of spherical carbogenic nanoparticles and the same organic fluorophore synthesized at 180°C. Quantum yields, however, decreased to 0.15 (compared to 0.50 from 180°C); and the emission behavior became excitation wavelength dependent. Higher reaction temperature was prone to have more carbogenic core; while lower temperature formed more organic fluorophore and greater fluorescence intensity (Figure 3.10). When carbogenic core/amorphous carbon had higher content of C-Dots, such as at 300°C, the quantum yield decreased to 0.04 (compared to 0.50 from 180°C and 0.15 from 230°C). In terms of element composition, higher temperature correlated to higher carbon element percentage and lower hydrogen percentage.
Other than trying to study the composition and synthetic mechanism, more synthetic materials are being tested. Earlier in 2012, Mohapatra et al.\textsuperscript{15} used an ordinary starting material to synthesize C-Dots using hydrothermal-pyrolysis method. The material is almost readily available in everyone’s daily lives: orange juice. Pulp-free orange juice was mixed with ethanol and heated at 120°C for 150 min in autoclave. No strong acid or surface passivation was needed. After purification with repeated solvent extraction, and repeated centrifugation, highly fluorescence C-Dots were obtained. They had average size of 1.5 – 4.5 nm. Quantum yield is 0.26 (quinine sulfate as reference standard). These C-Dots’ emission spectra were excitation wavelength dependent.

Later in the same year, microwave techniques have come to researchers’ attention. Wang et al.\textsuperscript{26} heated a solution containing citric acid and urea in a domestic 740 W
microwave for 5 min to synthesize C-Dots. The solution turned into dark-brown clustered solid, which became C-Dots after 20 min centrifugation (3000r/min). The formation of C-Dots was confirmed by TEM, AFM, XRD, Raman, $^{13}$C NMR, and FTIR. It is very interesting to see that the fluorescence was quenched when written on glass, metal, silicon or plastic substrate, but enhanced on paper. Quantum yield increased from 0.14 to 0.40. Due to its excitation wavelength dependency of emissions and its low toxicity, this C-Dots had great potential as biocompatible multi-color fluorescent ink (Figure 3.11).

![Figure 3.11](image)

Figure 3.11 (a) C-dot coated bean sprouts under day light, 340, 420, and 500 nm excitation (from left to right); (b) C-Dots marks on human skin; (c) C-Dots fluorescent fingerprints on filter paper at 420 nm excitation.
Since C-Dots are often mentioned to be ideal successors of semiconductor nanoparticles, such as quantum dots (QDs), C-Dots needed to match their marvelous fluorescence ability. Yang et al. conducted a systematic experiment using bottom-up synthesis to optimize the quantum yield. Citric acid and ethylenediamine were mixed in different molar ratios dissolved in water, and heated at 150, 200, 250 and 300 °C for 5 h in an autoclave. The brown-black product was then dialyzed for purification. Optimized quantum yield can reach up to 80% when 1 molar ratio of citric acid and 4 molar ratio of ethylenediamine were autoclaved at 200°C for 5 h. C-Dots not only were the ideal candidate to replace toxic semiconductor nanoparticles for bio-imaging, they were also sensitive to metal ions. Fe\(^{3+}\) can quench the C-Dots, thus C-Dots could also be used as metal sensor (Figure 3.12).

Figure 3.12 (a) Fluorescence quenching in the presence of Fe\(^{3+}\) ions (0–300 ppm); (b) Fluorescence intensities chart of C-Dots after the addition of different metal ions. (c) Fluorescence optical images of aqueous C-Dots after adding Fe\(^{2+}\) and Fe\(^{3+}\) ions. The on/off fluorescence was achieved by oxidation.
3.2.3 Popular Precursors: Citric Acid and Ethylenediamine

Among many precursors, citric acid and ethylenediamine (EDA) have became one of the most common combinations since 2012.\textsuperscript{27} Citric acid (CA) is a non-toxic small molecule with three carboxylic groups, which naturally present in numerous citrus fruits. It is often used as carbon precursor because of its low carbonization temperature (200°C).\textsuperscript{28} It is suggested that hydroxyl group, carboxyl group, and amine are indispensable in synthesizing C-Dots with decent QY.\textsuperscript{7} Other carbon sources, such as glucose, Tris,\textsuperscript{29} and sodium citrate,\textsuperscript{7} have been tested against citric acid, yet the quantum yield significantly dropped. CA is readily available, even from grocery store, and very affordable (less than $10 per lb), which makes it a popular carbonaceous precursor for C-Dot synthesis and research.

Ethylenediamine (EDA) is a basic organic molecule, which has two primary amines. It serves as a common nitrogenous source and the surface passivation agent for many C-Dots syntheses. Researchers have shown that nitrogen doping contributes significantly to the decent QY of C-Dots.\textsuperscript{29,30} Chemical structures play a major role in optimizing the QY of C-Dots, to be specific, the degree of nitrogen substitution, numbers of amines, and the length of alkyl chain. Some examples are listed below.

Primary amines can dope or passivate the C-Dots better than secondary or tertiary amines. It has been shown that EDA outperforms hexamethylene tetramine (HMTA), triethylamine (TEA), and diethylene amine (DEA). C-Dots were able to form from all four nitrogenous precursors. However, among all the amines mentioned above, C-Dots synthesized from EDA produced highest nitrogen element percentage by XPS, as well as the highest QY.\textsuperscript{27,29} Primary amines are less structurally hindered, and more approachable
when C-Dots formed during the synthesis. In short, primary amines are more desired over secondary or tertiary amines as C-Dots nitrogenous precursors and surface passivation agents.

The number of amines inside the chemical structures matters as well. Both being primary amines, EDA and ethanol amine differ only in the number of amines. Experiments shown that EDA produces higher QY than its monoamine analogue, ethanol amine,\(^29\) although C-Dots were formed in both cases.

EDA is a relatively small molecule and its alkyl chain length also contributes to the decent QY of its C-Dots. Using the same experimental condition, 1,4-butanediamine (BDA) was tested against EDA since BDA has two more carbon than EDA. The result turned out to be that shorter alkyl chain is more ideal.

As small and readily available molecules, CA and EDA keep the reaction facile and play perfect roles as carbon source and N-dopant precursor/passivation agent for many C-Dots.

### 3.3 Photoluminescence Mechanism

Though many chemical precursors and many synthetic routes have been explored, the photoluminescence origin is still debatable. A few hypotheses have been developed over the past decade. Namely, the free zigzag edge effect, synergistic effect of both molecular state and core state, and fluorescent by-products.
3.3.1 Free Zigzag Edge Effect

“Free zigzag edge” refers to the free hanging pieces from the edges of graphene and its derivatives. Graphene material has been a very hot topic since its Nobel Prize in Physics in 2010. Graphene sheet has been then used to synthesize C-Dots as a starting material via bottom up approach. Nano sized C-Dots (9.6 nm in diameter) were formed from micrometer-sized graphene sheets after a serial of oxidation, de-oxidation, and dialysis processes. The photoluminescent (PL) behavior is excitation wavelength dependent and pH dependent. The PL mechanism was suggested due to its carbene-like free zigzag sites. The hypothesis was supported by its pH-dependent PL behavior. When the free zigzag sites of C-Dots were protonated in acidic environment, the FL was then completely quenched. When pH later increased to alkaline conditions (pH = 13.0), PL was fully restored and this process was reversible.

3.3.2 Synergistic Effect of Molecular State and Core State

Precursors other than graphene were more popular. For many bottom-up carbonization synthesized C-Dots, the most commonly proposed PL mechanism is the combination of both molecular state and core state.

When carbon precursors (i.e. citric acid and ethylenediamine) were autoclaved at high temperature (200°C), a carbogenic core was formed and the sequential oxidation and reduction processes coated the carbon core with various surface groups. These surface groups contributed to the molecular state of C-Dots. The characteristic excitation wavelength dependency was contributed to the various surface functional groups. When the C-Dot samples were exposed to high-power UV light, the photochemical structures
on the surface were therefore destructed and the QY was reduced drastically to 20%. The remaining 20% of PL activities was due to the core state. The observation supported the hypothesis that the PL of C-Dots consisted not only from molecular state but also from core state.

This hypothesis was further explored in 2014.\textsuperscript{32} The C-Dots were synthesized using common precursor citric acid and ethylenediamine and found to have an asymmetric PL emission peak. It turned out to be two peaks instead, which included one strong PL peak (c.a. 445 nm) from molecular state as well as one from core state (c.a. 420 nm). The PL emission from core state was relatively weaker, and was hidden by the strong 445 nm emission peak. When PL emission of the molecular state was quenched by ferric ions or hydroxyl radicals; however, the PL lifetime remained the same. This indicated that the other state (i.e. core state) was dominating the PL lifetime properties while PL behavior of molecular state was suppressed.

Therefore, the synergy of multiple PL states are accepted and used to explain the PL mechanism.\textsuperscript{33}

### 3.3.3 Fluorescent By-product(s)

Along with the hundreds of articles published on C-Dots, some discrepancies occurred on PL behaviors of C-Dots. Excitation wavelength dependency was an important character to define C-Dots. Nonetheless, researchers began to report that some C-Dots could also have emission peaks that are excitation wavelength independent.\textsuperscript{28,34-36}

To better explain the discrepancies, Song et al.\textsuperscript{37} designed some deliberated synthetic pathways to show that the PL of C-Dots was, in fact, due to a combination of
surface state, molecular state, and a bright blue fluorescent molecule, called IPCA (imidazo[1,2-a] pyridine-7-carboxylic acid, 1,2,3,5-tetrahydro-5-oxo-), and its derivatives (Figure 3.13).

Figure 3.13 A simplified diagram of the relationship between precursors (citric acid and ethylenediamine) and C-Dots in a hydrothermal synthesis.

IPCA and its derivatives were generated from the C-Dots synthesized as by-products when experimental condition permits (Figure 3.14). When the reaction was carried out hydrothermally at 140°C, IPCA would be generated. IPCA was bonded onto the carbon core as well as other functional groups, and emitted strong blue PL. The exceptional excitation wavelength independence PL behavior was indeed due to the organic fluorophore, IPCA.
Figure 3.14 Synthetic scheme for IPCA (a) and its optical characterizations (b & c).

These one-pot synthesized C-Dots have a synergetic effect of three fluorescence origins, i.e., the carbon core state, molecular state, and a covalently attached organic fluorophore.
Chapter 4 Synthesis of C-Dots using Acids and Ethylenediamine as Precursors

4.1 History of C-Dots Discovery

The development of new carbon-based nanomaterials has been the focus of many research groups during the past couple decades. Some materials being developed include, but are not limited to, fullerenes,\(^3\)\(^{38,39}\) carbon nanotubes,\(^4\) nanodiamonds,\(^5\) and carbon dots (C-Dots).\(^6\) C-Dots is relatively recent; however, it has received an increasing amount of attention due to the favorable properties these nanoparticles possess. Carbon dots offer notable advantages over many similar nanoparticles, such as water solubility,\(^4\)\(^{36,44}\) tunable emissions,\(^4\) ease in synthetic approach, and low toxicity.\(^9\)

C-Dots were first discovered unintentionally in 2004 from an experiment purifying single-walled carbon nanotubes (SWCNTs).\(^3\) Through agarose gel electrophoresis, SWCNTs have been separated into multiple bands and the fastest-moving segments were found to be fluorescent nanoparticles, which were then named C-Dots. Since then, C-Dots have drawn a great attention due to their non-toxicity,\(^9\) biocompatibility,\(^9\) photoluminescence properties, and optical stability.\(^9\) These unique properties of C-Dots enable many promising potential applications, not only as biosensors,\(^9\) but also as environmental pH and hydrogen sulfide sensors in vitro,\(^9\) photocatalysts,\(^9\) electron donor in photo-reduction of metals,\(^9\) and protein concentration indicators.\(^9\)

Other synthesis methods have been well investigated, and the C-Dots products were characterized.\(^9\) In 2006, Sun et al.\(^9\) reported the first synthesis and characterization of carbon dots through acid oxidation of laser ablated graphite powder and cement, followed by passivation with polyethylene glycol. Inspired by Sun, other
research groups reported many C-Dots synthesis techniques, consisting of hydrothermal/solvothermal approaches,\textsuperscript{53-55} microwave synthesis,\textsuperscript{27,56,57} and electrochemical oxidation. Another appealing quality of carbon dot synthesis is the wide spectrum of starting materials that can be implemented, including inexpensive organic compounds/solvents,\textsuperscript{58} food products,\textsuperscript{59-61} and waste.\textsuperscript{18}

While a wide range of techniques have been applied to produce carbon dots, the nature of the generated products vary greatly. For example, Zhu et al.\textsuperscript{7} reported an increase in quantum yield (QY) from 20.9 to 80.6\% , simply by changing the ratio of the starting materials. Many strategies have been developed to increase the luminescence of these particles, with the most effective approach to date being doping with nitrogen.\textsuperscript{34} Carbon dots are typically used in solution form, however, some groups further modify the carbon dots by embedding or conjugating them to other particles to enhance functionality.\textsuperscript{62,63} With most carbon dot research of late being focused on increasing the optical capabilities, little work has been done on the material properties.

In this chapter, a facile one-pot synthesis of a carbon dot gel material (Figure 4.1) is reported. The carbon dot gel is formed without the use of gelating agents. Addition of citric acid to boiling ethylenediamine yields a viscous, gel-like carbon dot product, with physical and chemical properties dependent upon the reaction conditions. The temperature and the duration of the reaction were shown to affect both the optical and material properties.
Figure 4.1 New carbon dots produced from citric acid and ethlenediamine forms a gel material upon short reaction times, which possess excitation depended emission. A) Carbon Dot Gel; B) Upturned Gel; C) UV shortwave excitation; and D) Excitation with laser pointer.

4.2 Material Characterizations of C-Dots

The synthesis of C-Dots have been extensively studied, which can be divided into two major groups: “top-down” approaches such as arc-discharge, laser ablation, electrochemical exfoliation, and “bottom-up” approaches such as hydrothermal methods, supported synthetic, and microwave methods. The “top-down” methods refer to the breaking-down of the carbon source material, then forming C-Dots; the “bottom-up” methods usually involve a molecular precursor that is later oxidized by strong acids. Among all the synthetic methods mentioned above, one-pot hydrothermal syntheses have attracted the most attention due to its efficiency and easiness. Different starting materials for hydrothermal synthesis have been tested, including some readily available carbon sources, as common as orange juice or milk. Although many starting materials for C-Dots synthesis have been explored, the mechanisms and the structures of C-Dots still remain unknown. The photoluminescence (PL) of C-Dots is usually
excitation wavelength dependent;\textsuperscript{45} however, there are studies reported C-Dots have excitation wavelength independent PL.\textsuperscript{13,34} The mechanisms of C-Dots PL properties have a few hypotheses that still require further verifications. The most accepted hypotheses are the combination of surface state and core state.\textsuperscript{11,34,67-70}

As a promising nanomaterial, C-Dots still have areas to be thoroughly explored: their exact chemical structure and commercial applications. Carbon dots have received great attention as a result of the favorable optical properties these nanoparticles possess. While much work has been done toward enhancing the optical properties, little investigation has been done on the material properties of these nontoxic nanoparticles. Understanding the correlation between material properties and its commercial application would be beneficial.

Herein, a simple one-pot procedure for producing a highly viscous, thixotropic carbon dot gel material from citric acid and ethylenediamine is presented. The material properties are explored in detail. Carbon dots possess tunable emissions that are excitation-wavelength dependent and are ideally viscous materials. Detailed exact chemical structures are interpreted based on MS data.

Additionally, it came to our attention that some researchers have proposed QY enhancement methods, such as reducing the surface functional group\textsuperscript{71} or optimizing reaction conditions;\textsuperscript{7} however, few publications have mentioned a cross-reference for QY with a second reference. Using a cross-reference can ensure the accuracy of QY. In this chapter, the correct QY measurement and calculation are deliberated.
4.2.1 Preparation of C-Dots: Temperature and Reflux Time

For the preparation of gel-like C-Dots, three different temperatures and two different reaction times were chosen. The temperatures utilized were 120, 150, and 180°C with reaction times being either 10 min or 5 h. The procedure for producing C-Dots gel-like involves first, heating ethylenediamine to near boiling point in a round bottom flask. Once boiling starts to occur, citric acid is added directly at a 1:14 molar ratio (citric acid:ethylenediamine) (Scheme 2). A jacketed condenser is added and the reaction is heated to the desired reaction temperature and refluxed for either 10 min or 5 h, depending upon earlier reaction time. Upon completion, excess ethylenediamine is evaporated off, leaving behind a thick, viscous yellow gel. The produced gel is then dissolved in distilled water and placed into dialysis bag (MWCO 100 - 500 Daltons) in water for 2 days, changing the water every 4 to 10 h. Upon completion of dialysis, the C-Dots can be dispersed in water or isolated through evaporating off the water. The syntheses were triplicated to ensure the reproducibility.

The products were assessed with UV/vis spectroscopy, spectrofluorimetry, mass spectrometry, Fourier transform infrared spectroscopy (FTIR), atomic force microscopy (AFM), dynamic light scattering (DLS), Zeta potential and X-ray photoelectron spectroscopy (XPS).
Scheme 2 Proposed reaction scheme for the synthesis of carbon dot gel through the reflux of citric acid and ethylenediamine.

4.2.1.1. UV-visible Spectroscopy

The properties of the gel-like C-Dots, produced from citric acid and ethylenediamine, were found to be dependent upon the reaction conditions. The UV-visible absorption spectra of the carbon dots dispersed in water (Figure 4.2) shows broad absorption in the range of ~250 - 400 nm, with two prominent peaks possessing absorption maxima at approximately 285 and 335 nm, respectively. The absorbance maximum at 285 nm corresponds to $\pi - \pi^*$ transition of sp$^2$ carbon, and the absorbance peak at 335 nm is assigned to both n - $\pi^*$ character of C=O transition and $\pi - \pi^*$ charge transfer. The absorption band at 335 nm shows a net increase in absorption as a function of reaction time, as well as a blue shift of approximately 10 nm. While a minimal effect of reaction temperature is observed at 10 min, a large effect is observed after 5 h. The
band at 285 nm appears to decrease as a function of time, as the peak disappears at 180 °C from 10 min to 5 h.

Figure 4.2 Absorption spectrum for carbon dots prepared from citric acid and ethylenediamine at different temperatures reacted for A) 10 min, and B) 5 h.

4.2.1.2. Fluorescence Spectroscopy

The broad range of absorbance of the C-Dots led to a range of possible excitation wavelengths for photoluminescence, with a maximum photoluminescence found at an excitation of 360 nm (Figure 4.3A). The C-Dots possess tunable excitation-dependent emission wavelengths and an overall red shift, with the emission shifting from 347 nm at an excitation of 280 nm to an emission of 445 nm at an excitation of 400 nm (Figure 4.3B). Quantum yields of luminescence were calculated using quinine sulfate as a reference. The C-Dots produced at 180°C for 10 min, not only portrayed the most ideal gel-like properties, but they also possess a quantum yield of 38.7 %, the highest of all the prepared samples (Table 4.1).
Figure 4.3 A) Emission spectra of C-Dots gel-like (180 °C, 10 min) dispersed in water at various excitation wavelengths, and B) normalized emission spectra.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Reaction Time</th>
<th>Quantum Yield of Luminescence (%)</th>
</tr>
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<tr>
<td>120</td>
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<tr>
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<td>5 h</td>
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<tr>
<td>150</td>
<td>10 min</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>5 h</td>
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</tr>
<tr>
<td>180</td>
<td>10 min</td>
<td>38.7</td>
</tr>
<tr>
<td></td>
<td>5 h</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Table 4.1 Quantum yield of luminescence (in reference to quinine sulfate) of the C-Dots prepared at various temperatures and reaction times.
4.2.1.3. Quantum Yield Measurement

The quantum yield was calculated via the luminescence measurement, using quinine sulfate as a reference standard, with the following equation: \( \Phi = \frac{\Phi_R \times \frac{I}{I_R} \times \frac{A_R}{A} \times \frac{\eta^2}{\eta_R^2}}{\eta} \), where \( \Phi \) is the quantum yield, \( I \) is the measured integrated emission intensity, \( \eta \) is the refractive index of the solvent, \( A \) is the optical density, and the subscript \( R \) refers to the reference standard. Lucigenin is used as secondary reference against quinine sulfate. Lucigenin \((5 \times 10^{-7} \text{ M})\) is dissolved in pure water and prepared fresh before the measurement, and \(1 \times 10^{-6} \text{ M} \) quinine sulfate is dissolved in 0.1 M sulfuric acid aqueous solution (refractive index = 1.346). Using the quantum yield of quinine sulfate in 0.1 N H\textsubscript{2}SO\textsubscript{4} (QY = 0.60),\textsuperscript{73} the quantum yield of lucigenin in aqueous solution is then calculated to be 0.65, with 3% error from the literature value, 0.67.\textsuperscript{74}

It is important to follow strictly the experimental conditions or criteria to assure correct determination of quantum yield. Cross-calibration is very crucial for reliable QY measurements. For successful cross-calibration, the following criteria should be taken into consideration: (1) the two reference chemicals chosen are expected to have similar excitation and emission wavelengths so that the absorbance and fluorescence spectra overlap as much as possible; (2) include the correct solvent refractive indices when calculating the QY; (3) carefully plan the sample concentration range so that the absorbance value is kept between 0.02 and 0.05 (to avoid concentration effects, e.g. self-quenching, re-absorption of the emission, internal reflection, etc.); (4) careful handling of chemicals and solvents (e.g. avoid chloride ions), all the glassware need to be scrupulously clean and solvents must be of spectroscopic grade and checked for background fluorescence before the experiment.
4.2.1.4. X-ray Photoelectron Spectroscopy

The C-Dots gel-like was prepared for XPS analysis via pipetting the gel onto a Ta foil sample holder and outgassing in a vacuum desiccator at 1 Torr at 120°C until the solvent evaporated. The sample was then mounted onto the PHI 560 antechamber that was evacuated using a Balzers 300 L/s turbomolecular pump at room temperature (21±1°C) and then inserted into the auto chamber. Complete coverage of the sample holder was assured by the absence of Ta 4f peaks in the survey spectrum (Figure 4.4). High resolution XPS scans were obtained for the C 1s, O 1s and N 1s core levels. Calibration was performed using Au 4f7/2 and Cu 2p3/2 core levels for Au and Cu reference foils at 84.0 and 932.7 eV, respectively. The system pressure did not exceed 2x10⁻⁸ Torr during XPS scans.

![XPS survey scan of carbon dots showing X-ray excited Auger emission of O KVV, and N 1s, O 1s and C 1s core levels.](image)

Figure 4.4 XPS survey scan of carbon dots showing X-ray excited Auger emission of O KVV, and N 1s, O 1s and C 1s core levels.
XPS was performed with the purpose of analyzing the surface composition of the C-Dot gel. A survey scan was performed, which is shown in Figure 4.4, and revealed three distinct regions of interest emanating from C 1s, O 1s, and N 1s core levels. Regarding the C-Dots gel-like synthesized at 180°C for 10 min, the C 1s level shows three peaks relating to three oxidation states of carbon located at 284.9, 286.8 and 288.6 eV (Figure 4.5A).

![XPS core level shifts of C-Dots. (A) C 1s, (B) O 1s and (C) N 1s orbitals](image)

These peaks were attributed respectively to sp²-hybridized C, C-O-H, and O-C=O bonding within the gel-like C-Dots, respectively, which have also been observed in XPS scans of C-dots performed by other groups. Integrated peak area ratios of carbon
reveal an 8.2:1.0:4.1 proportion respectively of the sp²-hybridized C : [C-O-H] : [O-C=O] groups present in the C-Dots structure. The O 1s level shows two peaks which were assigned to 531.2 and 532.6 eV (Figure 4.5B); these peaks were attributed to C=O and C-O-H groups respectively, also consistent with the XPS literature values for C-Dots. The C 1s binding energy at 288.6 eV is believed to emanate from carboxylate groups, also consistent with XPS carbon dot literature values. Integrated peak area ratios of the oxygen level reveal a 1.24:1 ratio of the C=O:C-O-H groups. The N 1s level shows a single peak at 399.9 eV (Figure 4.5C), which we attribute to –NH₂ and –NH₂ groups, matching XPS literature values for these binding energies. The atomic percent composition from the C 1s, O 1s and N 1s core levels, normalized to the orbitals respective atomic sensitivity factors, was found to be 81.7 % C, 16.0 %O and 2.3 % N, respectively.

4.2.1.5. Rheology

The viscosity profiles are similar for all of the prepared samples, which all exhibit Newtonian behaviors at low to intermediate shear rates, and non-Newtonian behaviors at higher shear rates. The highest viscosity is observed for C-Dots material prepared at 120 °C for 10 min, which exhibit an order of magnitude higher viscosity than those prepared at 180°C for 5 h (Figure 4.6A).
Figure 4.6 (A) Flow behavior of C-Dots material prepared at different temperatures with different reaction times at 25°C. (B) Preset Shear rate profile and the corresponding time-dependent viscosity (thixotropy) of carbon dot materials prepared at the temperature of 180°C at 10 min (blue) and 5 h (orange).

The trend in viscosity is likely a result of hydrogen bonding. It is proposed that the C-Dots material is formed from hydrolysis under basic conditions of the carboxylic acid residues of citric acid with ethylenediamine, producing a large network of hydrogen bonds between adjacent –COOH and –NH₂ (Figure 4.7).
Figure 4.7 Proposed reaction scheme for the synthesis of gel-like C-Dots via reflux of citric acid and ethylenediamine.

The rheology of the produced C-Dots material was further investigated by observing the thixotropic behavior. The carbon dots prepared at 10 min reaction times displayed excellent thixotropy (Figure 4.6B), as the viscosity regains its original value back during the rest period (i.e., zero applied shear stress state [introduced by the pink trace]). The structure recovery ratio (SRR) was calculated using Rheoplus Software and was found to be 96.10% after 10 sec and 99.98% after 60 sec. While the samples prepared at 10 min exhibit thixotropic behavior, the carbon dots produced from 5 h reaction times do not show thixotropy (Figure 4.6B [orange trace]). Creep and creep recovery tests ascertained that the C-Dots gel-like material is ideally viscous.

Thixotropic properties are ideal for inks and wall paints, because thixotropic fluid will form a uniform layer right after applying and will resist further flow. Further sagging or paint smear could be avoided using this type of ink or paint. Moreover, blood is also an example of thixotropic fluid. Blood has many shear thinning colloids and responds
instantaneously to shear rate change. The thixotropic property allows the viscosity of blood to decrease with shear rate increase in order to enable the bio-system running under different circumstances. As the rheology data shown, C-Dots have thixotropic properties at high shear rates, which makes C-Dots a great potential for ink, paint or even blood medicine related applications.

As such, higher temperatures and longer reaction durations would yield products with a greater extent of hydrolysis, and in turn, a reduction in the amount of –COOH and –NH₂ residues available to participate in hydrogen bonding. Decreased hydrogen bonding will result in a decline in viscosity. As observed in Figure 4.6A, as viscosity decreases both as a function of temperature and the reaction time.

The viscous nature of the C-Dots gel-like material was further probed through measuring the crossover frequency. The dynamic moduli $G'$ and $G''$ are highly frequency dependent. Between angular frequency of 0 and 240 rad/sec, the viscous modulus $G''$ is predominant over elastic modulus $G'$. At this phase, the fluid, in this case C-Dots, is considered to be viscous fluid. After the crossover frequency at 240 rad/sec, $G'$ override $G''$, meaning elasticity takes over viscosity. This crossover frequency depends upon the reaction time of the prepared C-Dots material. Carbon dots prepared at reaction times of 10 min possess higher crossover frequencies than those prepared at 5 h. The crossover frequency is inversely related to the longest relaxation time ($t_{max}$) and represents the time taken by the C-Dots particles to disentangle. It has been previously shown that the disentanglement time of samples containing polymeric liquids experience an increase of relaxation time as the chain length, and in turn, the molecular weight of the polymer
increase. It is likely that a similar trend is occurring in this C-Dots material. As the reaction time is elevated, the extent of ethylenediamine reacting with citric acid will experience an increase, leading to materials with greater mass, and therefore greater relaxation time (Figure 4.8). The crossover frequencies also suggest that C-Dots could exhibit viscoelastic behavior under certain circumstances. Being a viscoelastic material, the fluid will dissipate energy/heat when a load is applied therefore could be used as commercial vibration isolators, noise absorbers, or shock absorbers.

![Graph](image)

Figure 4.8 Variation of storage and loss moduli as a function of applied angular frequency at 25 °C for samples prepared at temperature of 180 °C with (A) 10 min and (B) 5 h reaction times.

4.2.1.6. Electrospray Ionization (ESI) Mass Spectrometry (MS) of C-Dots Gels

ESI-MS data obtained on the C-Dots material prepared at 180°C for 10 min (Figure 4.9), and detailed interpretation in Table 4.2.
Figure 4.9 ESI-MS spectrum using the negative ion mode for the C-Dots gel prepared at 180°C for 10 min.

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Table 4.2 Suggested assignment of prominent peaks from ESI-MS.

4.2.1.7. Atomic Force Microscopy (AFM)

Carbon dot samples were prepared for AFM analysis by first dispersing the C-Dots gel-like in water to dilute the sample and to help mitigate the difficulty in transporting the C-Dots material. A single drop of the dilute C-Dots was placed onto a mica slide and the water is allowed to evaporate (~2 h). Once the slide is dry, the measurements were carried out using the tapping mode. Extracted profiles were reproduced several times to ensure the results reflected the sample as a whole.

Atomic force microscopy (AFM) was able to characterize the carbon dots, with a size distribution between 1 and 6 nm (Figure 4.10). The C-Dots material are uniformly dispersed, with no aggregation observed. The average height was found to be between 4 and 5 nm, by taking the mean of several extracted profiles from several different carbon dot samples.
Figure 4.10 AFM images for C-Dots material. A) AFM image, B) Profile extraction, and C) Extracted profile height distribution.

4.2.1.8. Conclusion

In conclusion, a facile procedure for generating highly viscous carbon dots has been presented. The carbon dots can be produced in a matter of minutes under rather mild reaction conditions, with minimal purification necessary. These carbon dots are found to be ideally viscous and form a gel possessing excellent thixotropic behavior when prepared at short reaction times. While some groups have attempted to produce highly
viscous carbon dots through the addition of gelation agents, this method is produced in one-pot with only citric acid and ethylenediamine. The specific ratio of ethylenediamine to citric acid was found to be pivotal, as a change in ratio could yield superior quantum yield, but this occurs at the expenses of materialistic properties as the ratio presented in this manuscript was the only one found to produce a gel. While the carbon dot gel does not possess the highest quantum yield presented for carbon dots, at 38.7% it is luminescent enough to be applied. The carbon dot gel was shown to have favorable properties in writing, which could be advantageous in multicolor printings (shown in Chapter 6).

4.3 Different Acids as Building Blocks Precursors

4.3.1 Selection of the Building Blocks

In order to study further on the fluorescence behavior and origin, the experimental condition and reactants need to be controlled carefully and varied systematically. Citric acid based C-Dots (use citric acid as a precursor) is one of the most fluorescent systems (QY ca. 94%) reported. As mentioned before in Chapter 3, citric acid and ethylenediamine are the most commonly used precursors. Among the carbon sources test, glucose, Tris, and sodium citrate, citric acid produces highest QY. Among the nitrogen dopants, ethylenediamine was proven to be the best candidate because of its degree of nitrogen substitution (primary amine), number of amines (highest), and alky chain length (shortest).
Nonetheless, there is no systematic study on the chemical structural change of citric acid like ethylenediamine. Citric acid has only been compared with other random carbon sources. Herein, a systematic study of 5 acid building blocks is reported here.

Citric acid is a weak organic tricarboxylic acid having six carbon atoms. One may wonder the importance of the number of carbon atoms and the number of carboxylic groups in the preparation of C-Dots. To investigate the significance of carbon atom numbers and carboxylic groups in the modules we chose the following acids: formic acid (FA), oxalic acid (OA), aspartic acid (AA), glutamic acid (GA), and citric acid (CA). The five acids are selected in the order of increasing numbers of carbon atoms and carboxylic groups. Using the same experimental condition to produce the high QY C-Dots, all the acids were heated with ethylenediamine at 180 °C for 4 h. The products were then assessed with UV/vis spectroscopy, spectrofluorimetry, mass spectrometry, Fourier transform infrared spectroscopy (FTIR), atomic force microscopy (AFM), dynamic light scattering (DLS), Zeta (ζ) potential and X-ray photoelectron spectroscopy (XPS). A second reference, lucigenin, has been chosen to ensure the accuracy of QY calculation method.

4.3.1.1. UV-visible Spectroscopy

In general, C-Dots show a characteristic broad shoulder in the UV region (200 - 400 nm) with a tail extending to the visible range (400 - 500 nm), which is the case for our synthetic C-Dots. The broad UV shoulder and the extending tail are mostly due to the starting material (Figure 4.11), and the peaks formed after synthesis are characteristic of C-Dots. C-Dots synthesized using a formic acid (FA) building block has two peaks
namely at 280 and 350 nm (Figure 4.11), while C-Dots using oxalic acid (OA) as building blocks have only one peak at 345 nm. C-Dots from aspartic acid (AA) building blocks have no obvious peak. C-Dots from glutamic acid (GA) building blocks have two peaks at 281 and 328 nm, and C-Dots synthesized from citric acid (CA) produced one peak at 324 nm. The C-Dots synthesized, except for AA, have both \( \pi - \pi^* \) transition (below 300 nm) and \( n - \pi^* \) transitions (above 300 nm). For instance, FA has only one carbon atom and the C-Dots synthesized from FA produced a \( n - \pi^* \) transition at 350 nm; meanwhile, CA, on the other hand, has six carbon atoms and produced a \( n - \pi^* \) transition at 324 nm. The UV peak (\( \pi - \pi^* \) transition) is not sensitive to number of carbon or carboxyl groups; however, \( n - \pi^* \) transition are. \( \pi - \pi^* \) transition corresponds to \( sp^2 \) dominated carbon core; while \( n - \pi^* \) transition indicates the carbonyl/amine surface functional groups.\(^{30}\)
Figure 4.11 UV-vis spectra of C-Dots prepared from a molar ratio 14 : 1 ethylenediamine: five different acids (formic acid, oxalic acid, aspartic acid, glutamic acid, and citric acid) synthesized at 180°C for 4 h at pH 9 ~ 10. FA, OA, AA, GA C-Dots concentrations are 10 mg/mL; and the CA C-Dot sample concentration is 100 μg/mL.

4.3.1.2. Fluorescence Spectroscopy

The photoluminescence spectrum data (Figure 4.12) show a very interesting trend that all the C-Dots prepared from formic acid, oxalic acid, aspartic acid and glutamic acid are excitation wavelength dependent, while the C-Dots from citric acid are excitation wavelength independent. The C-Dots from aspartic acid building block doesn’t have an absorption peak, and therefore no shift. The C-Dots prepared from FA, OA, GA and CA have a Stokes shift of 165, 90, 161 and 122 nm (or 6099, 5997, 5488 and 8442 cm⁻¹).
(calculated from Table 4.3), respectively. No obvious trend of Stokes shift is observed among the five C-Dots prepared from various acids.

a) Formic Acid 180 °C

![Normalized Intensity vs. Wavelength graph for Formic Acid 180 °C]

- 280 nm
- 300 nm
- 320 nm
- 340 nm
- 350 nm
- 360 nm
- 380 nm
- 400 nm

slit: 5, 5 nm

b) Oxalic Acid 180 °C

![Normalized Intensity vs. Wavelength graph for Oxalic Acid 180 °C]

- 280 nm
- 300 nm
- 320 nm
- 340 nm
- 350 nm
- 360 nm
- 380 nm

slit: 5, 5 nm
L-aspartic Acid 180 °C
0.333 mg/mL
slit: 5, 5 nm

Normalized Intensity
Wavelength (nm)

Glutamic Acid 180 °C
0.25 mg/mL
slit: 5, 5 nm

Normalized Intensity
Wavelength (nm)
Figure 4.12 Photoluminescence spectra of C-Dots prepared from a molar ratio 14:1 ethylenediamine: acid. a) formic acid, b) oxalic acid, c) aspartic acid, d) glutamic acid, and e) citric acid synthesized at 180 °C for 4 h at pH 9–10.
Table 4.3 UV-vis absorption and photoluminescence peak positions of C-Dots synthesized from a molar ratio of 14:1 ethylenediamine:acid for all five different acids at 180 °C for 4 h; listed in the order of increasing numbers of carbon atoms. Measurements were done in water at 25 °C. All the photoluminescence bands are excitation dependent, except the C-Dots synthesized from citric acid and ethylenediamine heated at 180 °C for 4 h, which is excitation independent.

### 4.3.1.3. Quantum Yield

All the quantum yields of five C-Dots are measured using the same methodology in Chapter 4 using quantum yield calculation \( \Phi = \phi_r \times \frac{l}{l_r} \times \frac{A_r}{A} \times \frac{n_r^2}{n_r^2} \).

As for the results, quantum yield of C-Dots prepared using citric acid (QY= 0.05) is the highest, followed by glutamic acid building block (QY= 0.03), aspartic acid (QY=0.01), and oxalic acid (QY≈0) (Table 4.3) with the exception of formic acid.

<table>
<thead>
<tr>
<th>C #’s</th>
<th>Molecule Name</th>
<th>Formula/MW</th>
<th>UV/vis</th>
<th>Photoluminescence</th>
<th>QY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \lambda_{abs} ) nm</td>
<td>( \lambda_{em} ) nm</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Formic Acid</td>
<td>CH(_2)O(_2) 46.03g/mol</td>
<td>280/350</td>
<td>445</td>
<td>3.2</td>
</tr>
<tr>
<td>2</td>
<td>Oxalic Acid</td>
<td>C(_2)H(_4)O(_4) 90.03g/mol</td>
<td>345</td>
<td>435</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>Aspartic Acid</td>
<td>C(_4)H(_7)NO(_4) 133.11g/mol</td>
<td>-</td>
<td>456</td>
<td>1.4</td>
</tr>
<tr>
<td>4</td>
<td>Glutamic Acid</td>
<td>C(_3)H(_9)NO(_4) 147.14g/mol</td>
<td>281/328</td>
<td>442</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>Citric Acid</td>
<td>C(_6)H(_8)O(_7) 192.14g/mol</td>
<td>324</td>
<td>446</td>
<td>5.1</td>
</tr>
</tbody>
</table>
(QY=0.03). It is suggested that the increasing number of carbon atoms from the building blocks are related to higher quantum yield of the CD products.

4.3.1.4. X-ray Photoelectron Spectroscopy (XPS)

XPS was performed on each of the carbon dots: FA, OA, AA, GA, CA using the sample preparation and scanning procedures reported previously.\textsuperscript{84} The C 1s orbital was deconvoluted into two chemical oxidation states. A 70-to-30 % Gaussian-Lorenztian lineshape was used to curvefit the XPS data using a Shirley background subtraction.\textsuperscript{85} Atomic percentages from the C 1s, O 1s, and N 1s core levels, normalized according to their atomic sensitivity factors,\textsuperscript{78} are summarized in Table 4.5. The N 1s orbital at 399.1-399.4 (2.4-2.7) eV (with full-width at half maxima in parentheses) is consistent with –NH or –NH\textsubscript{2} groups within the C-Dots structure.\textsuperscript{77,86} Similarly, the O 1s orbital at 530.9-532.5 (2.2-2.6) eV denote C-OH groups within this same makeup.\textsuperscript{86}

<table>
<thead>
<tr>
<th>Atomic %</th>
<th>C</th>
<th>O</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>33.4</td>
<td>65.5</td>
<td>1.1</td>
</tr>
<tr>
<td>OA</td>
<td>71.3</td>
<td>11.1</td>
<td>17.6</td>
</tr>
<tr>
<td>AA</td>
<td>65.4</td>
<td>12.0</td>
<td>22.6</td>
</tr>
<tr>
<td>GA</td>
<td>77.6</td>
<td>10.8</td>
<td>11.6</td>
</tr>
<tr>
<td>CA</td>
<td>68.1</td>
<td>22.4</td>
<td>9.5</td>
</tr>
</tbody>
</table>

Table 4.4 XPS atomic percent composition of C-Dots.
Table 4.5 XPS binding energy data of carbon dots synthesized from formic acid, oxalic acid, aspartic acid, glutamic acid, and citric acid.

<table>
<thead>
<tr>
<th>Orbital</th>
<th>Formic Acid (FA)</th>
<th>Oxalic Acid (OA)</th>
<th>Aspartic Acid (AA)</th>
<th>Glutamic Acid (GA)</th>
<th>Citric Acid (CA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 1s</td>
<td>284.9 (2.3)</td>
<td>284.9 (2.5)</td>
<td>284.9 (2.5)</td>
<td>284.9 (2.6)</td>
<td>284.9 (2.5)</td>
</tr>
<tr>
<td></td>
<td>287.4 (2.8)</td>
<td>287.1 (2.8)</td>
<td>287.3 (2.0)</td>
<td>287.5 (2.0)</td>
<td>287.3 (2.4)</td>
</tr>
<tr>
<td>O 1s</td>
<td>532.5 (2.5)</td>
<td>531.2 (2.4)</td>
<td>530.9 (2.2)</td>
<td>531.6 (2.6)</td>
<td>531.4 (2.6)</td>
</tr>
<tr>
<td>N 1s</td>
<td>399.4 (2.7)</td>
<td>399.3 (2.7)</td>
<td>399.1 (2.5)</td>
<td>399.2 (2.6)</td>
<td>399.5 (2.4)</td>
</tr>
</tbody>
</table>

The carbon dots OA (71.3 %), AA (65.4 %), GA (77.6 %), and CA (68.1 %) have similar percent composition of the C 1s orbital but a significant drop is seen for FA carbon dots (33.4 %) (Table 4.4). The O 1s orbital showed relatively similar percent composition in OA (11.1 %), AA (12.0 %), and GA (10.8 %) to be the lowest followed by CA (22.4 %), second highest, and FA (65.5 %) with the highest composition. The nitrogen percent composition is lowest in FA (1.1 %), second highest in GA (11.6 %) and CA (9.5 %), and the highest in OA (17.6 %) and AA (22.6 %). A bar chart of XPS elements data is showed in Figure 4.13. All C-Dots, but not the one using FA as building block, have very similar carbon atomic components despite they started with different carbon atom numbers. No obvious trend is observed regarding oxygen or nitrogen atomic percentage either. C-Dot formation appears to be non-stoichiometric.
Figure 4.13 XPS atomic percent composition based on the C 1s, O 1s and N 1s core levels.

Integrated peak areas of the C 1s orbital showed similarities in composition between AA, GA, CA, and OA. The atomic percent composition for FA, however, stood out for having low nitrogen content.
Figure 4.14 Stackplot of XPS data shows that C 1s XPS core levels of C-Dots synthesized using various amino acids. Solid line envelope at 284.9 eV denotes the C-C structure within the C-Dots. Dashed line envelope at ca. 287.3 eV denotes the C-O-C structure.

Further examination of the C 1s level (Table 4.6) shows desorption of ether-like C-O-C in GA and OA in comparison to AA, CA, and FA. The C 1s core level envelopes were fitted at binding energies of 284.9 (2.3-2.6) and 287.1-287.6 (2.3-2.8) eV (Table 4.5), denoting the C-C, and ether-like C-O-C structures, respectively. For simplicity, we refer to these peaks as C1 and C2, respectively. Peak C1 denotes the oxidation state assigned to C-C. The relative ratios of non-oxidized (C1) to oxidized carbon (C2) were compared. Carbon dots made of CA and FA had non-oxidized to oxidized ratios (based
on integrated peak area ratio of C1/C2) of 4.3 and 4.8, respectively, while those made of AA, OA, and GA had a ratios of 5.1, 7.5, and 7.8, respectively. The data indicate that ether groups are mostly desorbed when the carbon dots are made of OA or GA.

The surface carbon element percentage of the five carbon dots are predicted to increase with increasing number of carbon atoms the starting carbon material. The result doesn’t exclude the possibility of having increasing carbon composition inside of the C-Dots spheres along with the increasing number of carbon atoms from the starting materials. However, XPS data (Table 4.4) shows no such correlation on surficial carbon composition.

<table>
<thead>
<tr>
<th>Assigned Structure</th>
<th>Formic Acid (FA)</th>
<th>Oxalic Acid (OA)</th>
<th>Aspartic Acid (AA)</th>
<th>Glutamic Acid (GA)</th>
<th>Citric Acid (CA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-C</td>
<td>82.7</td>
<td>88.3</td>
<td>83.6</td>
<td>88.6</td>
<td>81.1</td>
</tr>
<tr>
<td>C-O-C</td>
<td>17.3</td>
<td>11.7</td>
<td>16.4</td>
<td>11.4</td>
<td>18.9</td>
</tr>
</tbody>
</table>

Table 4.6 Relative area percentages of C 1s peaks of carbons dots that synthesized from formic acid, oxalic acid, aspartic acid, glutamic acid, and citric acid

4.3.1.5. Zeta Potential

The surface functional groups determine most of the C-Dots characteristics, therefore Zeta (ζ) potentials are informative in terms of surface functional group behavior. Bovine serum albumin (BSA) is used to confirm that the method is valid before measuring C-Dots. All the C-Dots showed (Table 4.7) negative Zeta potential values, which confirmed that C-Dots are covered with a negative charge in aqueous solution. The
negative charges are likely due to carboxyl and hydroxyl groups on the C-Dots surfaces. However, no obvious trend is observed with increasing the number of carbon atoms or increasing the number of carboxyl groups in the acids building blocks.

<table>
<thead>
<tr>
<th>BSA</th>
<th>FA</th>
<th>OA</th>
<th>AA</th>
<th>GA</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10.8±0.9</td>
<td>-22.9±3.5</td>
<td>-14.6±4.1</td>
<td>-11.2±2.4</td>
<td>-15.7±6.6</td>
<td>-20.7±4.1</td>
</tr>
</tbody>
</table>

Table 4.7 Zeta potential (mV) of C-Dots synthesized from a molar ratio 14:1 of ethylenediamine : acid at 180°C for 4 h. The acids are formic acid (FA), oxalic acid (OA), aspartic acid (AA), glutamic acid (GA), and citric acid (CA). Data measured at 25 ± 1 °C. The concentration of bovine serum albumin (BSA) was 10 mg/mL in PBS, and the concentrations of all the C-Dots were 5 mg/mL in 0.05 M KCl solution.

4.3.1.6. ATR-FTIR

ATR-FTIR spectrum is shown below (Figure 4.15). The analysis of ATR-FTIR of the C-Dots showed the hydrophilicity by the O-H stretching band (3343 cm⁻¹), N-H stretching band (3280 cm⁻¹), C-H stretching band (2922 cm⁻¹), and C-O stretching band (1371 cm⁻¹); and the conjugation system is confirmed by C=C stretching band (1555 cm⁻¹) and C=O stretching band (1647 cm⁻¹).
Figure 4.15 ATR-FTIR data of the C-Dots prepared from a molar ratio 14 : 1 of ethylenediamine with each of the 5 acids at 180°C for 4 h: formic acid (FA), oxalic acid (OA), aspartic acid (AA), glutamic acid (GA), and citric acid (CA).

4.3.1.7. Mass Spectrometry

Mass spectra are helpful when determining synthesis products. Since experimental condition play a vital role in C-Dots synthesis, a little variation in synthetic condition could lead to completely different products. Despite many researchers have reported similar routes, the products might vary under slightly different solvent,
temperature, or reactant ratios. Mass spectra are very informative tools to confirm the final products.

Formic acid is the smallest building block selected among the five acids, and it has only one carboxyl group. Formic acid has a molecular weight of 46.03 and ethylenediamine has a molecular weight of 60.10. Mass spectrum suggested the majority of the products are dimers along with various polymers.

Figure 4.16 Mass spectrum using negative ion mode of C-Dots from formic acid and ethylenediamine at 180°C for 4 h.
For oxalic acid based C-Dots, the mass spectrum indicated the presence of mainly dimer products (MW 174.20) of oxalic acid (MW 90.03) and ethylenediamine (MW 60.10) and various polymers.

Figure 4.17 Mass spectrum using negative ion mode of C-Dots from oxalic acid and ethylenediamine at 180°C for 4 h.
For aspartic acid based C-Dots, the mass spectrum showed the major products are monomers (MW 175.19) of aspartic acid (MW 133.10) and ethylenediamine (MW 60.10) and dimers without an amine group (MW 202.26).

![Mass spectrum](image)

Figure 4.18 Mass spectrum using negative ion mode of C-Dots from aspartic acid and ethylenediamine at 180°C for 4 h.
The building blocks, glutamic acids, have two carboxyl groups, which can form polymers with ethylenediamine. The MS suggests that glutamic acids and ethylenediamine reacted and formed monomers without an amine group (MW 173.22) and dimers (MW 231.20).

Figure 4.19 Mass spectrum using negative ion mode of C-Dots from glutamic acid and ethylenediamine at 180°C for 4 h.
Citric acid is the largest building blocks selected among the five acids, and it has three carboxyl groups and one hydroxyl group. The MS shows that the products were mostly dimers (MW 276.29) along with various polymers.

Figure 4.20 Mass spectrum using negative ion mode of C-Dots from citric acid and ethylenediamine at 180°C for 4 h.

Under the experimental conditions, the products formed from five acid building blocks and nitrogen source ethylenediamine are mainly dimers with some monomers and polymers according to mass spectra. Compared to the other four acid building blocks, citric acid based C-Dots have much higher MW products, which indicates higher content of polymers and presence of carbogenic cores. This helps to explain the excitation wavelength independent and dependent emission behaviors of citric acid based C-Dots.
To summary, the absorbance that due to $\pi$ - $\pi^*$ transition is not sensitive to the carboxyl group numbers, but n - $\pi^*$ transition blue shifts when the carboxyl group number increases. PL emission behaviors, however, are not related to carboxyl group numbers. Only citric acid produced C-Dots that were excitation-wavelength independent emission, which is comparable to chemical fluorophores. QYs of C-Dots products are proportional to the numbers of carbon atoms from the carbon sources. Based on XPS elemental analysis and Zeta potential data, C-Dots surface conformation is non-stoichiometric.
Chapter 5 Synthesis of Tryptophan-based C-Dots

5.1 Rationale

Previous C-Dots had been synthesized using carbon sources that have no fluorescence. Herein, we propose to synthesize using a fluorescent amino acid, tryptophan, as a carbon source to achieve a higher quantum yield C-Dots while maintaining its biocompatibility. Tryptophan as a precursor has one more favorable characteristic; it can cross the blood-brain barrier (BBB) through the LAT1 transporter.

Two types of carbon dots were synthesized and reported in this chapter using tryptophan and two different nitrogen dopants, viz. urea and ethylenediamine. Ethylenediamine is commonly used in the preparation of C-Dots as a nitrogen dopant because it has been shown to increase the quantum yield of photoluminescence.\textsuperscript{90-92} While it is toxic on its own, it has been shown that C-Dots prepared with ethylenediamine are not toxic at low and moderate concentrations.\textsuperscript{93,94} Ethylenediamine is commonly used with citric acid to synthesize C-Dots.\textsuperscript{90,92,93,95} Replacing citric acid with a molecule that is already fluorescent should increase the quantum yield of the C-Dots formed under these conditions. Tryptophan was chosen as the carbon precursor for this purpose.

Tryptophan is one of twenty essential amino acids and essential for many biological processes including the production of melatonin and serotonin.\textsuperscript{96} Because it is required in the brain, there is a specialized form of transport to cross the BBB referred to as LAT1, which is composed of the glycoprotein CD98.\textsuperscript{97,98} Due to this property, it would be advantageous to incorporate C-Dots, which has tryptophan present on the surface, make it possible for the C-Dots to cross the BBB through the LAT1 transporter.
Urea is used as a nitrogen dopant, which is a less toxic alternative to ethylenediamine. Since it is a solid, unlike ethylenediamine, a solvent is necessary to carry out the reaction with a solid carbon precursor. The carbon dots resulting from ethylenediamine appear promising for the traversal of the BBB. UV/vis spectroscopy, spectrofluorimetry, Fourier transform infrared (FTIR) spectroscopy, transmission electron microscopy (TEM), and atomic force microscopy (AFM) images are reported below.

5.2 Spectroscopic and Microscopic Characterization

Tryptophan-based C-Dots were synthesized from tryptophan and ethylenediamine in a 1:14 molar ratio (1.0923 g and 5.0 mL respectively) and heated, with spin, to 180°C for 4 h. The reaction was carried out under argon to prevent the oxidation of the ethylenediamine. The product was then taken to a rotovap machine to remove excess ethylenediamine. A yellow gel-like liquid remained after this process. To remove excess monomeric tryptophan, gel electrophoresis was performed. A 10 % agarose gel was used and a 0.1 M Bis-Tris buffer was used to buffer the gel (pH = 5.9). The tryptophan control was stained with Coomassie Blue and could be seen moving only slightly forward (Figure 5.1), since the pH of 5.9 is just above the isoelectric point of tryptophan (5.89).
Figure 5.1 Image for CD-Urea during electrophoresis under white light (top) and under UV light (bottom).

Under the light of a UV lamp, the blue luminescence of the tryptophan-based C-Dots could be seen moving backward, toward the negative electrode. After 15 minutes, when the luminescence had completely separated from the wells (Figure 5.2), the gel was cut, dispersed in deionized water, and centrifuged at 3000 rpm for 20 minutes.
The supernatant was taken and filtered through a 0.2 µm syringe filter and lyophilized to obtain a gel-like substance (CD-EDA) with a very pale yellow color similar to previous reactions using ethylenediamine. This same process was repeated for a reaction with tryptophan and urea. The same molar ratio of 1:14 was used (0.5462 g and 2.2486 g for tryptophan and urea, respectively) and combined with 6 mL of deionized water with a resistivity of 18.2 MΩ·cm. The evaporation step was omitted because there was no liquid precursor. This yielded a gel-like substance (CD-Urea), which was less yellow than the corresponding product with ethylenediamine.

The electrophoresis separation provided some insights into the nature of the two different C-Dots: CD-EDA and CD-Urea. The luminescent band in both products moves
backward (toward the negative electrode), in the opposite direction of tryptophan alone, suggesting that the surface of the C-Dots is positively charged, possibly from protonated amine groups. In addition, CD-EDA can be seen in a very narrow luminescent band while CD-Urea is much more widely distributed (Figure 5.1 and Figure 5.2). Two possibly explanations for this difference in the distribution of C-Dots are: the size distributions of the respective samples and differences in uniformity of the surfaces of the C-Dots in the two samples. The optical properties and size measurements give insight into these options.

The absorbance of the product before purification shared very similar traits with tryptophan and after electrophoresis the tryptophan absorbance peak at 280 nm could be seen, but the absorption below this peak was higher than in tryptophan and the peak was shifted to closer to 210 nm (Figure 5.3a and c).
The peak at 280 nm can be attributed to the n - π* transition for C=O, as in tryptophan. The higher energy peak at 210 nm can be attributed to the π - π* transition for the aromatic sp²-hybridized carbons provided by tryptophan. The absorption moiety at 280 nm indicates that some tryptophan remains attached to the surface of the C-Dots. Based on the relative heights of the two peaks for each sample, it would appear that there is more tryptophan in CD-EDA than CD-Urea. This could potentially create a more homogenous surface state on CD-EDA. When the fluorescence spectrum is analyzed,
greater differences can be seen between CD-EDA and CD-Urea (Figure 5.3). The photoluminescence spectrum for CD-EDA shows little shifting of the emission with increasing excitation wavelength, whereas CD-Urea shows a more common amount of shifting of emission. The absorbance spectrum shows there is more tryptophan left on the surface of CD-EDA (Figure 5.4 and Figure 5.5) and this may create a more homogenous surface that results in less shifting of the emission.

Figure 5.4 Absorbance spectrum for CD-EDA
Additionally, the maximum emission in CD-EDA comes from 325 nm excitation as opposed to 375 nm in CD-Urea. The blue shifting of the maximum excitation wavelength comes from the higher presence of tryptophan (maximum excitation of 290 nm). This also creates a very sharp drop off in emission intensity above 425 nm in CD-EDA. This photoluminescence data agrees with the electrophoresis images (Figure 5.1 and Figure 5.2).

The AFM and TEM images in Figure 5.6 and Figure 5.7 show the sizes of the synthesized carbon dots. The mean size from TEM measurements for CD-EDA and CD-Urea was 6.2 and 4.1 nm, respectively. The sizes obtained from AFM are in general agreement with this. The size distribution for CD-EDA is much wider than for CD-Urea, which is not what would be expected based on the electrophoresis images. The size
distribution data confirms that the distribution seen in electrophoresis resulted from the surface state and the size effect was minimal for the distribution.

Figure 5.6 (a) AFM image for CD-EDA, (b) AFM height profile for CD-EDA, (c) TEM image for CD-EDA, (d) TEM histogram for CD-EDA.

Figure 5.7 (a) AFM image of CD-urea, (b) AFM height profile for CD-Urea, (c) TEM image for CD-urea, (d) TEM histogram for CD-Urea.
Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) were used to examine the surface of the C-Dots. The peaks and functional groups are summarized in Table 5.1, and the spectra in Figure 5.8, Figure 5.9, and Figure 5.10.

<table>
<thead>
<tr>
<th>XPS</th>
<th>FTIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1s</td>
<td>-O-H</td>
</tr>
<tr>
<td></td>
<td>C-O</td>
</tr>
<tr>
<td>O1s</td>
<td>amide C=O</td>
</tr>
<tr>
<td></td>
<td>C=ONH(_2)</td>
</tr>
<tr>
<td>N1s</td>
<td>-C=N-</td>
</tr>
<tr>
<td></td>
<td>N-H stretch</td>
</tr>
<tr>
<td></td>
<td>N-H bend</td>
</tr>
<tr>
<td></td>
<td>C-N</td>
</tr>
</tbody>
</table>

Table 5.1 Comparison and classification XPS and FTIR characteristic peak

<table>
<thead>
<tr>
<th>Element</th>
<th>Percent of CD-Urea</th>
<th>Percent of CD-EDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>60.6 %</td>
<td>55.0 %</td>
</tr>
<tr>
<td>Oxygen</td>
<td>39.2 %</td>
<td>44.7 %</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.2 % (59.4 % imine and 40.6 % amine/amide)</td>
<td>0.3 % (49.1 % imine and 50.9 % amine/amide)</td>
</tr>
</tbody>
</table>

Table 5.2 Elemental analysis from XPS data.
Figure 5.8 FTIR spectrum for CD-EDA

Figure 5.9 FTIR spectrum for CD-Urea
Figure 5.10 XPS core levels of O 1s, N 1s and C 1s orbitals of C-dot gel for (A) CD-EDA and (B) CD-Urea

All of the functional groups shown in the XPS data are correlated with the FTIR peaks (Table 5.1). The FTIR spectra describe similar functional groups of the two different types of C-Dots, which are similar to other C-Dots with perhaps the exception of the missing carboxylic acid group. The main difference between the two spectra can be seen in the two peaks just above 1600 cm\(^{-1}\). The higher energy peak indicates either C=O of an amide group or C=N and the slightly lower energy peak is assigned to the N-H stretch of amines or amides. The lower intensity of the higher energy peak in CD-EDA indicates a lower presence of imine or C=O groups. From the elemental analysis
summary given by XPS (Table 5.2), it can be seen that there is 10 % less surface imine
groups in CD-EDA, which would explain the differences in the FTIR spectra.

The absence of carboxylic groups from both spectra is interesting because of the
presence of the absorbance that is characteristic of tryptophan. This can be explained by
the formation of peptide bonds. There is a ratio between carboxylic groups and amine
groups of 1:28 in the starting materials, so the probability of peptide bond formation
between the two groups is high and would explain the disappearance of the carboxylic
groups and the appearance of amide groups. The imine group could result from the
isomerization of the indole group in tryptophan to an indolenine group. This is
normally not the most favorable isomer, but the carbon dot core and surface could
stabilize the structure and provide the C=N functional group seen in the XPS spectra. The
oxygen in the samples results from the oxidation of the carbon dot surface and not the
oxidation of the amine groups during the reaction (due to the protected atmosphere by
argon). This oxidation results in a very strong alcohol peak (ca. 3300 cm$^{-1}$) in both
samples.

Because of the low percentage of nitrogen present in CD-EDA and CD-Urea, 0.3 %
and 0.2 % respectively, the only nitrogen present is likely the result of the tryptophan
present in the surface of the C-Dots. CD-EDA has a higher percentage of heteroatoms in
general than CD-Urea (Table 5.2). The greater presence of functional groups on the
surface as well as the higher presence of tryptophan (Figure 5.4 and Figure 5.5) creates
the higher uniformity seen in the electrophoresis process and the fluorescence spectra for
CD-EDA when compared to CD-Urea.
The two CD samples synthesized in this study show the desired property of having tryptophan present on the surface of the C-Dots after purification. The CD-EDA sample shows the added advantage of having a more uniform surface state. This sample needs to be tested with cells for a toxicity analysis and with zebrafish to confirm the ability of the C-Dots to cross the blood-brain barrier. The possibilities in imaging or drug delivery require further investigation into these CD samples, particularly CD-EDA.
Chapter 6 Applications of C-Dots

6.1 Ink for Lithography and Printing

One particular area of application of thixotropic materials is drug delivery.\textsuperscript{80,81} Drug delivery systems are enhanced by materials expressing “smart” properties in which the material responds to external stimuli. The carbon dots were tested for “smart” properties, through observing the effects of pH and temperature. Upon exposure to pH above or below 7 to 8, the viscous properties of the material are destroyed and the gel cannot be reformed. Furthermore, the C-Dots gel is responsive to temperature, as the material is observed to shrink when cooled to 0°C, while it swells upon heating to 40°C. One major limitation of the carbon dot gel for a drug delivery system is its high dispersity in water. For these to achieve better biocompatibility they would need to be further functionalized.

Thixotropic materials can also be utilized as printing inks. The ink properties of this material were observed by writing with the material dispersed in water and observing under UV light (Figure 6.1). While various groups have shown the use of carbon dots in multicolor printing, to the knowledge of the authors, none have shown that the ink can be erased. As shown in Figure 6.1, the C-Dots material was written onto a glass slide and completely erased from the slide by simply rinsing briefly under running water.
Figure 6.1 C-Dots gel excited under UV light on A) a glass slide and B) glass slide after rinsing briefly with water.

In addition to write on a glass slide, C-Dots can also be used as ink for writing or printing on papers (Figure 6.2). After drying the C-Dots ink, the “U” pattern written on a filter paper was invisible to human eyes (Figure 6.3), but visible when under UV light. Based on the high fluorescence and low cost, C-Dots have a great commercial potential to be used for invisible/disappearing/black light reactive writing. To further explore the market potential for C-Dots, more experiments are done using real human hair, crayon, nail polish, etc.
Figure 6.2 C-Dots are dispersed in aqueous solution and written onto a piece of filter paper using a paint brush. Picture taken under 365 nm (left) and 400 nm UV light (right).

Figure 6.3 After drying, the written “U” is invisible under electric incandescent lamp to human eyes (Left: wet; right: after drying).
6.2 Commercial Potentials:

6.2.1 Hair Imaging Before and After

To illustrate the commercial potentials of C-Dots, fluorescent hair shampoo is used as an example. After immersing real human hair into C-Dots solution, the brown hair was then air-dried before was taken under UV lamp (Figure 6.4). Such C-Dots shampoo is bio-compatible and can be used to decorate hair in circumstances such as night clubs that use black light. Hair will glow bright blue under UV light.

![C-Dots “dyed” brown hair shining under 365 nm UV light.](image)

Figure 6.4 C-Dots “dyed” brown hair shining under 365 nm UV light.

Current commercial yellowish green fluorescent dyes (e.g. La Riche Direction, UK) are mostly thick gels, and can only be applied as conditioner (comb-apply, set for 15
min to 30 min before rinse), and cannot be combined with shampoo for use. This creates an extra step for the users compared to our one-step C-Dots fluorescent dye shampoo. Due to the high quantum yield of C-Dots and the use of affordable starting materials, the cost to fabricate the dye shampoo is low which makes our fluorescent shampoo competitive to any current products.
Chapter 7 Conclusions

The use of C-Dots has been a hot topic since 2004 due to their potential to replace its predecessor, quantum dots. As a benign starting material, carbon can produce decent quantum yield after careful design. C-Dots can handle diverse surface functional groups for further modifications or attachments thus have numerous biological applications. Various starting materials have been experimented, from carbon soot, citric acid, to milk, orange juice, and different experimental conditions have been explored, from arc discharge, electrochemistry, heating-stirring, to microwave.

Under certain experimental conditions, C-Dots formed are gel-like. No materialistic study has been done those C-Dots gel-like. A thorough rheological reported is presented. The samples are shown to be ideal viscous solution, which exhibits both Newtonian and non-Newtonian fluid behavior. At high shear rates, C-Dots posses the non-Newtonian behavior, which means that C-Dots are thixotropic under circumstances. The thixotropic property makes C-Dots a great potential for ink, paint or even blood medicine related applications. Additionally, the viscoelastic property of C-Dots also provides the insight of C-Dots being a potential shock/noise/vibration absorbing materials.

The mystery of fluorescence mechanism still remains debatable. In this thesis, a series of systematic experiments were carried out to provide some insight into the fluorescence mechanism of C-Dots.

Five different acid building blocks were chosen to synthesize C-Dots. The elemental analysis shows the high number of carbon atoms from the starting carbon precursor, i.e., the acid building blocks, the high carbon percentage will present in the C-Dots products. Nonetheless, no other trend was observed on its absorbance or
photoluminescence behavior, except that the absorbance peaks (n - \( \pi^* \) transition) red shift when carboxyl group numbers decrease. The report suggested that the number of carbon atoms or the number of carboxyl groups from the carbon precursor have few impacts on C-Dots fluorescence.

Furthermore, instead of most commonly used carbon source citric acid, an amino acid, tryptophan, was used because of its decent fluorescence ability and its BBB penetration capability. In terms of nitrogen dopant, ethylenediamine and urea were used. It turns out that C-Dots synthesized from tryptophan and ethylenediamine are more uniformed at the surface and have less excitation wavelength dependency. It suggested that ethylenediamine would be a better nitrogen dopant compared to urea, and tryptophan serve as a better surface attachment when uniform C-Dots are desired.

In addition to all the favorable characteristics of C-Dots, C-Dots have great commercial potentials, as ink for writing tool or entertainment for hair coloring. More insight and delicate researches are necessary to understand and explore the full picture of C-Dots before it can be utilized further in academia, medical field, and commercial markets.
Works Cited


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