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FLUORESCENT PARTICLES FROM
CONCENTRATED SUGAR SOLUTION

By

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A THESIS

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Abstract

Simple glucose solutions were heat treated in an attempt to produce carbon nanodots (CNDTs) with a monodisperse size distribution using a bottom-up approach. Absorption and fluorescence properties of the heat-treated solutions display remarkable similarity to CNDTs reported in the literature. However, particle-sizing and AFM measurements indicate the increasing fluorescence is accompanied by the growth of particles that are larger than most CNDTs discussed in the literature (a concentrated population of monodisperse 30 nm particles and a low concentration of much larger, roughly 500 nm, particles). A dialysis study, shows these larger particles are not responsible for the bulk of the optical properties but, rather the optical properties likely stem from molecular particles lesser than 8 nm that accompany the heating and caramelization of the sugar.
Acknowledgments

First, I would like to specially thank my advisor, Dr. David Sidebottom, for his fatherly guidance, immense patience and his heart-filled benevolence throughout my years at Creighton University. His guidance has been an inspiration to me and will continue to be. My next journey in physics is due to his leadership and foresight.

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Finally, my deep gratitude goes to my parents, family and friends whose name are inscribed in the pages of my experience. Their support physically, mentally and emotionally brought me to the end of this phase.
For my family and for Dr. David L. Sidebottom
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Chapter 1

Introduction

The emergence of quantum dots late last century opened opportunities for “zero-dimensional” nanostructures (nanostructures less than 100 nm in all three dimensions) in various fields like bio-imaging, photovoltaics, sensors and diodes. However, disadvantages in the use of these quantum dots in biological systems has promoted the development of alternative nanostructures based on carbon. For example, heavy-metal quantum dots are hazardous to the environment and to human health [1] and semiconductor quantum dots used in bio-imaging must be specially engineered to be less toxic to biological cells. In addition, materials required for the fabrication of several quantum dots are not as readily available as those required for carbon dots synthesis. As the sixth most abundant element in the Milky Way galaxy [2], carbon is useful and common on earth existing in various forms – as sugars, and as graphite for example.

1.1 Carbon dots

Carbon Nanodots (CNDTs) were discovered accidentally from the purification of one-walled carbon nanotubes in 2004 by Xiaoyou Xu and his fellow researchers [3]. Carbon dots are comprised of a nanocarbon core below 10 nm in diameter, and typically coated by a functionalized surface, which is of modified carboxylic functional groups. Figure 1.1 illustrates this description of carbon dots with nanocarbon cores spherical in shape coated by carboxylic and carbonyl functional groups. Depending on the nature of the functional group on the surface of the carbon dots, they exhibit different properties. This makes carbon dots useful in drug delivery and also for targeting and extracting analytes [4] – chemicals of interest in analytical procedures.
Carbon dots have a mixture of amorphous and crystalline structure with varying amounts of carbon-carbon \( sp^2 \) and \( sp^3 \) bonding depending on the method by which they were synthesized. Based on the degree of crystallinity, two nomenclatures for carbon dots are used: Carbon Quantum dots (CQD) or Carbon Nanodots (CND). Carbon Quantum dots have graphitic layers of crystalline carbon with a quasispheroidal shape that are quantumly confined. Quantum confinement results when a material is too small compared to the de-Broglie wavelength of an electron. The random motion of electrons in the miniaturized material becomes restricted, more like an electron in a box, thus, creating energy levels and increased band-gaps. On the other hand, carbon nanodots have non-crystalline or amorphous nanocarbon cores which do not show the quantum size effect. In general, the term carbon dots (C-dots) is used to refer to either of these [5].

Carbon dots are a good candidate for bio-imaging due to their low toxicity and ease of conjugating with biological cells. What makes them even more useful in biological imaging is their up-conversion property – in which the absorption of two infrared photons causes photoluminescence like an absorption of a UV photon would. Although the fluorescence emission of Carbon dots is weaker for longer wavelengths, up-conversion is useful for in vivo optical imaging, due to the increased fluorescence of C-dots relative to the auto-fluorescence of the background tissues. Therefore, there is a better signal-to-noise ratio [6].
Additionally, carbon dots do not rapidly photo-bleach. Photobleaching is the gradual fading of fluorescence by a fluorophore. The resistance of carbon dots to photobleaching was also investigated by Jaiwal et al who discovered that the fluorescence of the carbon dot they made decreased at a rate of 0.06% per minute compared to the 0.59% of a popular fluorophore, Rhodamine 101 [7]. Furthermore, carbons dots are both electron donors and electron acceptors under visible-light radiation. This makes them beneficial for photovoltaics, as they help delay electron-hole recombination due to their electron accepting ability. This electron-accepting and electron-donating property of carbon dots, together with photostability, up conversion features, and low-toxicity makes them an excellent candidate for photovoltaics and bioimaging.

1.1.1 Optical properties of carbon dots

Carbon dots typically absorb in the UV region with absorption peaks at around 230 nm and 280 nm [8, 9]. The two absorption bands represent electron excitation from the π-state of C=C present in the core to the corresponding excited states, π*, and excitation to the same excited state from surface energy levels [8], respectively. The energy level diagram in Figure 1.2 summarizes the absorption and emission property of carbon. This energy level diagram model was like-wisely used by [8] to explain the optical properties of carbon dots.

![Schematic diagram of electronic transition in carbon dot (especially CNDTs)](image)

Figure 1.2: Schematic diagram of electronic transition in carbon dot (especially CNDTs)
Carbon dots, when irradiated by UV light and visible light, emit bright and colorful photoluminescence covering the entire visible-to-near infrared spectral range. A key property that distinguishes them from simple fluorophores is that the photoluminescence of carbon dots is dependent on the wavelength of the exciting light [8, 9]. The dependence of the emission on excitation wavelength may be due to the distribution of differently-sized carbon dots and surface chemistry, or the development of surface traps [9]. Thus, photoluminescence color of carbon dots is mostly due to nature of its surface rather than the size of the dot [9]. This is quite different from that of semiconductor-based and heavy metal-based quantum dots in which size-confinement primarily dictates the photoluminescence. Thus, chemical modifications (such as the presence of some elemental atoms like pyridinic nitrogen, hydroxylation etc.) can affect emission spectrum of carbon dots [10]. The synthesis route by which a carbon dot is made also affects the nature of the surface functionalization/passivation of the carbon dot – passivation refers to a material becoming passive because of it being shielded by a protective layer.

1.1.2 Synthesis Methods of Carbon dots

Carbon dots are synthesized using two either a “top-down” approach or a “bottom-up” approach.

**Top-down approach:** involves the breakdown of larger carbon structures like carbon nanotubes, graphite, nano diamonds, carbon rods etc. into nanometer-sized particles. The top-down approach can be imagined like using a hammer to break apart large chunks of carbon. This hammering can be achieved through many techniques including high-powered laser ablation, arc discharge, electrochemical oxidation, or ultra-sound [11]. Most carbon dots formed by these top-down approaches retain their crystallinity and so possess a perfect \( sp^2 \) carbon structure in their core but lack an efficient band gap to produce fluorescence [9]. They therefore require further surface functionalization/passivation to enhance their photoluminescence.

**Bottom-up Approach:** involves the building of carbon dots from smaller precursors like polyethylene glycol, carbohydrates (e.g. sugars), citric acid and other organic sources. The use of microwaves, plasmas, thermal decomposition, and hydrothermal treatments have been used to build carbon dots “bottom-up” from organic sources [11]. Bottom-up approaches are simple methods of creating carbon dots that could allow carbon dots to be mass-produced provided the formation process can be controlled. An inherent difficulty is that bottom-up approaches generally result in carbon dots that are polydisperse in nature [9] consisting of a distribution of particle sizes. Also
1.2. Motivation

In 2010, Sidebottom and Tran [13] used dynamic light scattering to investigate the cryopreservation property of simple sugar solutions. In their study, they observed sugar aggregate into uniformly-sized particles whose mean size increased with increasing concentration of the aqueous sugar (at low temperature). As aqueous sugars are attracted through hydrogen bonds [14], the increase in concentration could promote the formation of more intermolecular bonding. Above 82.5 wt. % particles begin to cross-link thus forming a gel. This relationship is observed in Figure 1.3 for different simple sugar molecules.

Figure 1.3: Illustrates the dependence of well-defined cluster sizes on concentration (in wt.%) of aqueous sugar for various simple sugars.
1.2. Motivation

In Sidebottom’s earlier work on concentrated sugar samples [15], orange photoluminescence was seen to emanate from the samples while exposing the samples to 532 nm laser light used for dynamic light scattering analysis (DLS). To produce these concentrated samples, samples of lower initial concentration were dehydrated after filtration and developed a yellow tint. An investigation of the emission spectra at varying glucose concentration for a variety of excitation wavelengths [15] was conducted and some samples exhibited excitation-dependent emission similar to that reported by CNDTs. Figure 1.4 summarizes the result of the investigation. From the Figure 1.4, the intensity, normalized to the scattering from a control sample of polystyrenes in water, increased with increase in concentration. Also, shown by the inset, the peak emission shifts in proportion to the excitation [14] indicating that the photoluminescence is an excitation-dependent emission.

![Emission spectra for various sugar concentration](image)

Figure 1.4: Emission spectra for various sugar concentration (in mol.%) for a 460 nm excitation light. The broad peak near 530 nm increases with increasing concentration. Shown by the inset, the wavelength of the peak emission shifts in correspondence to the excitation wavelength. Adapted from [15].
1.3 Thesis Statement

The hallmark of CNDTs is a wavelength-dependent emission. Given that such emission was seen in concentrated glucose solutions and that these solutions have well-defined cluster sizes, can lower concentration solutions be cooked to produce similarly well-defined CNDTs? If so, what are their optical and morphological properties? In my study, I have examined glucose solutions at 20, 40, and 60 wt.% cooked (hydrothermally treated) at 80, 90, 100, 120, and 140 °C for varying times. These solutions were then characterized with regards to optical properties (absorption and fluorescence) and morphology (particle size via photo-correlation spectroscopy).
Chapter 2

Literature review

The ease of bottom-up approaches to making, and mass producing, carbon dots come with a loss in the control of the formation process [9]. This loss in the formation process exhibits itself in the morphology and size distribution of carbon dots due to the random conglomeration during the carbonization process [16]. Nonetheless, the existence of uncontrollable formation processes does not deny the occurrence of interesting properties observed in the morphological and optical properties of carbon dots made “bottom-up”. In this section, we look deeper into the morphological and optical properties of some carbon dots made from several organic origins.

2.1 Morphology

Sahu et al. [17] fabricated carbon dots through a one-step hydrothermal treatment of orange juice. The orange juice was hydrothermally treated at 120 °C for less than 150 mins. Two categories of particles were formed from the carbonation process: the first category is a collection of highly fluorescent monodispersed particles identified as carbon dots, and the second category are less fluorescent coarse particles (CP) that are ten times bigger than the carbon dots [17]. High-Temperature Transmission Electron Microscopy (HRTEM) images from the paper showed spherically-shaped carbon dots with size ranging between 1.5 nm to 4.5 nm. The functional groups on the surface of the carbon dots were observed to be C-O-O-H, C=O, and O-H groups [17]. The size range of the CPs was, between 50 - 60 nm. The presence of coarse particles is interesting as similarly-sized particles were discovered in the course of this work.

Looking further into carbon dots synthesized from materials associated with fruits, Muthukumarman et al. [18] synthesized carbon dots by hydrothermally treating odium banana peel xanthate.
A xanthate is a salt formed from a reaction between an alkoxide (a conjugate base of an alcohol) and carbon disulphide [19]. This reaction is usually done in the presence of potassium hydroxide or sodium hydroxide. Thus, Muthukumaran et al. made a xanthate from banana peel by mixing pieced banana peel (treated with sodium hydroxide) in stoichiometric amount with carbon disulphide. Thereafter, the banana peel xanthate was hydrothermally heated in an autoclave at 180 °C for 12 hrs. Although, the size of the synthesized carbon dots was not explicitly stated, the surface of the carbon dots had C-O-C, C=S and C-O-C-S groups [18].

Observing another case, Monte-Filho et al. [20] synthesized facile carbon dots from lemon and onion juices. Using the microwave bottom-up technique, measured amount of pulp-free orange, onion juice and ammonium hydroxide were mixed and microwaved at 1450 W for 6 mins. The resulting mixture, after cooling, was then centrifuged for 30 mins at 6000 rpm and after which dialysis and evaporation were conducted to extract and concentrate the carbon dots [20]. The size distribution of the carbon dots was between 4.23 – 8.22 nm with a mean diameter of 6.15 nm. The functional groups on the surface of the carbon dots are O-H, N-H, C-H, C=O, C=N, C=S, and C-S [20]. It is important to note that there may have been particles formed in the carbonization process that are 10 nm in size, but because of the larger particles trapped by the 1 KDa dialysis membrane used in the research was not further investigated, it is not possible to accurately tell.

The previous three cases did not show a case that used a passivation agent (which is basically any chemical compound that modifies the surface of the carbon dots in order to enhance fluorescence). Aji et al. used urea as a passivation agent in the synthesis of carbon dots from the pyrolysis of mangosteen [21]. Mangosteen peels were first preheated in distilled water at 70 °C and then mixed with urea and heated in a furnace at three different temperatures for 30 mins. The reason for heating at three different temperatures (200, 250 and 300 °C) was to observe the influence of temperature on carbon dots properties. Interestingly, the size varied for different carbon dots synthesized at different temperatures. The carbon dots made at 200 °C had a size range between 10 – 15 nm, for the ones created at 250 °C the size range was between 7 – 11 nm, and finally, the carbon dots made at 300 °C had size range between 2 – 4 nm. This implies that heating the mangosteen at higher temperatures resulted in smaller carbon dots. In all the three categories of carbon dots made from this process, the active functional groups were C-H, O-H, N-H, C=O, and C=C [21].

From the four cases of carbon dots synthesized bottom-up from common organic materials, it is observed that some synthesis paths create monodispersed particles, with some size range, while some other synthesis paths create a mixture of differently sized particles. It is also apparent that the chemistry of the constituent components affects the surface functionalization of the engendering
2.2 Optical Properties

The carbon dots from Sahu et al. had a broad absorption at 288 nm. The absorption at 288 nm is more prominent for the larger carbon particles (CP). Thus, a single broad absorption peak at 288 nm was observed for the two categories of particles gotten from the hydrothermal treatment of orange juice. The absorption was accredited to the presence of aromatic $\pi$ orbitals which were numerous in the CP [17]. With an excitation wavelength of 390 nm, the broad emission peak for the 455 nm for the carbon dot peaked and 474 nm for the CPs. The difference in emission peak was attributed to the variation in size with the energy gap decreasing with increase in size. Sahu et al, attributed the photoluminescence (PL) mechanism to be attributed to surface defects pointing out the dependence of emission on the wavelength of exciting lights.

With regards to single absorption peak, the carbon dots from the banana peel xanthate had an absorption peak at 226 nm. The absorption was attributed to the $\pi-\pi^*$ transition for the C=S bond [18]. Also attributed to the $\pi-\pi^*$ transition for the C=S bond is the PL with a peak of 477 nm for an excitation wavelength around 350 nm.

Testing for excitation-wavelength dependence from 290 to 400 nm, Monte-Filho et al. observed a red-shifting of the emission with an excitation at 340 nm having the most intense emission [20]. The presence of surface states was explained to cause the wavelength-dependent emission behavior. Also, the carbon dots they synthesized absorbed at 340 nm which is ascribed to the $\pi-\pi^*$ transition for the C=O bond. A shoulder at 280 nm was also observed and was attributed to the $\pi-\pi^*$ transition for the aromatic $sp^2$ domains.

Finally, the carbon dots from mangosteen peel had a main absorption at 369 nm and another around 405 – 415 nm [21]. The absorption spectra depended on the amount of urea used during the pyrolysis process and also on the temperature of pyrolysis: the absorption peak for carbon dots made at 200 °C was 412 nm, 250 °C was 389 nm, and 300 °C was 389 nm. Also, the emission peak at the pyrolysis temperature of 200 °C was 465 nm, 250 °C was 450 nm and 300 °C was 423 nm under a 365 nm excitation wavelength. This blue-shifting of the emission corresponds to the effect of particle size on emission. The PL and absorption mechanisms of the carbon dots were also attributed to the radiative electronic transition in $\pi$ orbital.
2.3 Summary

From these various examples, we see that while controlling formation processes in bottom-up processes can be challenging, some parameters can be varied to vary the nature of carbon dots produced. An example of a parameter-control procedure was seen in the procedure done by Aji et al. [20] in which temperature affected the size of the synthesized carbon dots. Irrespective of the fabrication routes taken to make these particles lesser than 10 nm in size, these particles, carbon dots, exhibit similar optical property discussed in chapter 1.
Chapter 3

Experimental Methods

In order to investigate if a change in concentration of sugar solutions affects the size of the engendering carbon dots fabricated through the hydrothermal bottom-up approach using simple sugar (glucose) as a precursor, three different concentrations of glucose solutions were heated for the same time duration at the same temperature (see Table 3.1). The process is repeated for different temperatures and photon correlation spectroscopy is used to analyze the size of the particles formed. The optical properties are analyzed using photoluminescence spectroscopy and absorption spectroscopy.

3.1 Sample Preparation

Appropriate weights of glucose were added to weighed deionized Millipure water in a beaker. For example, for a 60 wt. % glucose solution, 60 parts by weight of glucose was mixed with 40 parts by weight of deionized Millipure water. The mixtures were stirred with a magnetic stirrer at 50 °C until a transparent solution was obtained. The resulting transparent solutions were then transferred into a Pyrex media bottle (100 ml). The Pyrex bottle was sealed and placed in a temperature-controlled oven set to a certain temperature. At selected time intervals, the bottles were removed, stirred to produce a uniform mixture, and a small amount was transferred into a clean 20 ml vial. After each extraction, the media bottle was replaced into the oven for further cooking and later extraction of other samples. Depending on the length of time in the oven, the samples obtained ranged in color from colorless to yellow to dark brown [illustrated in Figure 3.1]. Table 3.1 below shows the time for extraction of samples at different temperatures and concentrations.
3.2 Dilution

After extraction, each vial is diluted with more deionized water to avoid strong absorption of 532 nm light so that photo-correlation spectroscopy could be performed. For less brown samples the ratio of dilution was 1 part of cooked sugar solution extracted to 1 part of Millipore water by volume. In browner samples, this dilution was insufficient as much absorption was noticed during extraction.

Figure 3.1: Photograph showing samples of 60 wt.% cooked at 120 °C extracted at 1 hour, 3 hours and 5 hours respectively (from the left and undiluted).

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Concentration wt.%</th>
<th>Time (hours) A</th>
<th>B</th>
<th>C</th>
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<td>2.5</td>
<td>3</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Table 3.1: Table showing times samples were collected at different temperatures
3.3 Measurements

3.3.1 Dynamic Light Scattering

Dissolved particles in solutions are constantly in random motion. When a laser light is shone on these particles, the electrons present in the atoms of these particles interact with the electric field of the incoming laser light. The electric field of the laser light drives the electrons to oscillate along its direction of polarization with the same frequency. The oscillating electric field therefore radiates electromagnetic waves outwards with circular wave fronts in the plane perpendicular to the direction of polarization. Due to the relative location of electrons on the atoms or molecules of the particle there will be phase difference between the waves from the electrons. Wave fronts from the electrons, therefore, interfere at the detector to form interference patterns similar to the double-slit interference pattern.

The different relative locations of the particles further cause additional interference of the electric field scattered by each particle at the detector. Since the particles in solution are constantly in random motion and diffusing through the entire volume of the solvent, the intensity of the detected light pattern at the detector fluctuates in correspondence to the diffusion rate of the particles (solute) in the solvent. Therefore, finding the rate at which these fluctuations change can give information on the diffusion rate of the particles, and hence, the size of the particles.

Proceeding with this process, the diluted sample is filtered with a 0.8 micrometer cartridge filter to remove unwanted dust particles. This is done to ensure a successful photo-correlation spectrum is obtained. Vials were placed in a cylindrical fixture and illuminated by vertically-polarized laser light with a wavelength of 532 nm. Scattered light at an angle ($\theta=36.9^\circ$) to the forward direction of the incident light was imaged onto a 50-micron pinhole located approximately 50 cm from a photomultiplier tube (EMI 9863B). This is shown in the photograph below.
3.3. Measurements

Figure 3.2: An image showing the optical setup to vertically polarize the 532 laser light. The yellow circle shows the hallowed cylinder in which the vials sit in, and the yellow square shows the 50-micron pinhole for collecting the scattered light to the photo-multiplier.

The photo pulses from the photomultiplier were converted to a digital signal and sent to a correlator. The correlator computes the intensity-intensity autocorrelation function, $C(t)$, of the signals which is related to the dynamic structure factor, $S(q,t)$ as:

$$C(t) = 1 + A_C |S(q,t)|^2$$  \hspace{1cm} (3.1)

Here $q = (4\pi n / \lambda) \sin(\theta/2) = 9.94\mu m^{-1}$ is the scattering wavevector, and $A_C = 0.81$ is the signal-to-noise correction value associated with the optical collection system. This correction value was obtained using a suspension of polystyrene spheres as a control where $S(q,t) = 1$ at $t = 0$. The dynamic structure factor gives information about the inter-particle correlations and how they evolve in time. In the instance of particles of different sizes undergoing diffusion,
3.3. Measurements

\[ S(q, t) = \sum \alpha_i \exp^{-D_i q^2 t} \]  \hspace{1cm} (3.2)

where \( D_i \) is the diffusion coefficient of a particle of size \( R_i \). Assuming the particles formed in the cooking process are spherical, the diffusion coefficient is related to the size of the particles, by the Stoke-Einstein relation:

\[ D_i = \frac{kT}{(6\pi \eta R_i)} \]  \hspace{1cm} (3.3)

where \( k \) is Boltzmann’s constant, \( T \) is temperature, and \( \eta \) is the viscosity of the liquid medium (Millipure water). Thus, photo correlation spectroscopy provides information about the size of the particles created in the cooking process.

3.3.2 Photoluminescence

In addition to light scattering measurements, each sample was transferred to a smaller vial and illuminated with a Superbright\textsuperscript{TM} LED that had a peak emission at 385 nm. Fluorescent light emitted from the sample and scattered light from the LED are collected at right angle by a fiber optic cable beneath the vial. The collected light is transmitted to a small grating spectrometer (USB4000). The spectrometer produces a spectrum of the collected light signals which was analyzed by the Ocean Optics software. The intensity of the obtained spectra was then normalized with respect to the scattered intensity of the excitation peak.
3.3. Measurements

Figure 3.3: Picture showing the actual setup used for the measurement of photoluminescence. The vial sits in a cylindrical holder while a LED light shines from the side of the holder. A fibre optic cable transmits light collected from the base of the cylinder to a USB4000 spectrometer.

3.3.3 Absorption

Samples, thereafter, were transferred into a quartz cuvette which was placed in a Cary UV-Visible spectrometer which recorded the absorption from 800 nm to 200 nm. Deionized Millipore water was used as reference to remove background absorption of the water only. Samples were diluted by consecutive amounts to determine their extinction coefficient via plots of peak absorption against concentration. For example, a 20 wt.% sample was tested for absorption. At the end of the first test, half volume of the sample was mixed with equal volume of Millipure water to get a concentration (10 wt.%) half the previous concentration. The new diluted was tested for absorbance. This procedure was repeated until absorption could no longer be observed. The extinction coefficients was then
3.3. Measurements

gotten from the slope of the straight line plot of the observed absorption intensities against the corresponding concentrations.
Chapter 4

Results

Results for photon correlation spectroscopy (PCS), Photoluminescence (PL) and UV-Visible Absorption are presented in this chapter. The data for PCS, PL and UV-Visible spectroscopy presented here where all carried out on all the samples made at 20 wt.%, 40 wt.%, 60 wt.% at 80 °C, 90 °C, 100 °C, 120 °C and 140 °C. The size of the particles formed from the hydrothermal treatment of the glucose solution is determined in the analysis of the PCS data while the optical properties of the particles are shown in the analysis of the PL and UV-Visible data.

4.1 Photon Correlation Spectroscopy Results

The diffusion of small monodispersed glucose clusters present in solutions prior to heat treatment give rise to a single fast relaxation as shown in Figure 4.1 by the black hollowed-circles. Since the correlation between the intensities of scattered lights from any two clusters depends on the distance between the clusters, the correlation decreases at a shorter time for small clusters. These particles diffuse at a fast rate of roughly $10 \mu s$. The three other profiles in Figure 4.1 for cooked glucose solution show two relaxations: a fast at about 10 μs and a slow relaxation at about 1 ms. The two relaxations are indicative of two different cluster sizes. The fast relaxation is coincident with that of the uncooked glucose solution and is presumed to arise from the autocorrelation between uncaramelized (uncooked) glucose clusters seen earlier. The second relaxation is at a slower time indicating the diffusion of much larger particles; large particles have a slower diffusion rate and, therefore, the correlation of scattered lights from these particles take a longer time to decrease. Analysis of particle size via-Stoke-Einstein relation (stated in chapter three) yields a particle size around 500 (±200) nm in diameter for this second relaxation.
Interestingly, there is no indication from the PCS that these large particles develop gradually through a series of smaller but increasing sizes. Rather, the slow relaxation appears at earliest time of sample heating with a size roughly two orders of magnitude larger than the glucose clusters and its amplitude increase suggests the abundance of the particles increases with reaction time. We call these very large particles “popcorns” for that reason.

Figure 4.1: Plot showing the development of large particles shown by the second relaxation at a longer time. The presence two relaxation times in the profiles of the heated glucose solution signifies the presence of two different particle sizes: the uncaramelized glucose clusters and the popcorns. The popcorns are roughly 100 times larger than carbon dots and the uncaramelized glucose clusters.

These same popcorn structures were seen in all samples of different concentrations regardless of heating temperature or reaction time.
In Figure 4.2 the hydrodynamic diameter determined from the slow relaxation of the PCS data is compared with the hydrodynamic diameter for glucose solution observed by Sidebottom and Tran [13]. In the plot, the popcorns are 100 times bigger than the glucose clusters and there are no intermediate particle sizes between the popcorns and the glucose clusters.

![Hydrodynamic Radius vs. Sugar Concentration](image)

**Figure 4.2**: Plot showing the comparison of the hydrodynamic radius between ordinary glucose clusters and popcorns formed from glucose solution heated at 140 °C. The orange dots are the hydrodynamic diameter from Sidebottom and Tran [13], while the blue and green dots are hydrodynamic diameter for glucose solution heated at 140 °C for 2 hrs. and 2.5 hrs. respectively. From the of the plot, it is apparent the popcorns are 100 times larger in size than the glucose clusters.

### 4.2 Photoluminescence Results

The general trend observed for the photoluminescence spectroscopy done on the heated glucose solutions is illustrated in Figure 4.3 for the example of a 40 wt.% sample heated at 90 °C. The
emission profiles for an excitation wavelength of 385 nm shows a peak at 500 nm with two shoulders at around 450 nm and 530 nm. The presence of shoulders in these emission profiles is unique as literature examples of carbon dots usually show a broad emission peak without any shoulders.

From the plot, the emission increases in intensity with cooking time without any apparent change in shape. That is, browner samples show more emission intensity than relatively less-brown samples as would be expected for an increasing density of popcorns. The metric used to ascertain the growth of the emission intensity for samples cooked at various times is the relative height of the peak around 500 nm with respect to the 385 nm excitation peak of the plot. In order words, the fluorescence spectra are normalized relative to the excitation peak. It was observed across all concentrations and temperatures that the fluorescence intensity increased with more heating time. Therefore, in correlation with the development of popcorns with heating time, the fluorescence increases in intensity.

Figure 4.3: Photoluminescence (PL) spectra of three samples cooked at 90 °C. The plot shows the increase in PL intensity with cooking time. The inset is a log scale to illustrate how spectra merely increase in intensity without change in shape.
4.3 Absorption Results

In correspondence with the increase in fluorescence with cooking time, a similar increase in absorption was observed for the heat-treated glucose solutions. Two absorption peaks at around 230 nm and 280 nm were observed as shown in Figure 4.4 in excellent agreement with the literature on CNDTs [9]. These peaks were sharper and more precise for higher concentration samples and for samples heat-treated for longer times. Furthermore, the ratio, $I_{280\text{ nm}}/I_{230\text{ nm}}$, of the intensities at 280 nm and 230 nm is not constant for samples of the same concentration heated at a particular temperature for different lengths of time. In contrast to the emission spectra, the absorption at 280 nm grows more rapidly than the absorption at the 230 nm with more heating time increase. Since the 280 nm absorption takes place from surface states (see Figure 1.2) its accelerated growth could be consistent with the observed growth in large (popcorn) particles that have greater surface area and, presumably, more available surface states. This illustrates the growth of the surface states with more heating time.
4.4 Summary

It is observed that from the result, hydrothermally-treating glucose solutions caused the development of large particles (popcorns) which increase quantitatively with more cooking time for all concentrations at different temperatures. Also, as cooking time increases the solutions become more brown. Additionally, the intensity of absorption and fluorescence emission from all samples at different concentrations cooked at different temperatures increased with increase in cooking time.

Figure 4.4: An absorbance profile of treat-treated 40 wt.% glucose solution at 140 °C. The profile shows two absorption peaks at around 230 nm and 280 nm. It is apparent from the plots that the intensity of the absorption at 280 nm grows at the expense of the absorption at 230 nm.
Chapter 5

Discussion and Additional Studies

5.1 Discussion

From our results, as the glucose solutions are hydrothermally treated for increasing durations, absorbance and photoluminescence emission increases in intensity as the samples become browner and more popcorns develop. Also, the absorbance for the 280 nm wavelength increases beyond that of 230 nm wavelength with cooking time. This is consistent with the idea that more surface states (surface area) being generated by large popcorn fosters the 280 nm absorption branch. From these results from PCS, photoluminescence and absorption results, it is appealing to conclude that the increase in the optical response of our heat-treated glucose solution is due to the presence of the large (popcorns) particles.

However, near the conclusion of our studies, a paper by Essner et al. appeared that cautioned about assuming that making ‘bottom up’ approaches only make CNDTs [12]. According to Essner et al., the bottom-up approaches to making CNDTs, like the hydrothermal method and microwave method, also produce molecular by-products (possibly, due to the caramelization process, in our case). The optical response of the CNDTs can be overshadowed by these molecular by-products making it difficult to draw concrete conclusions of what exactly produces the fluorescence and absorption that is observed.

In hindsight, we now recognize that PCS can be deceptive when very large particles are present. Since the intensity of scattered light by a particle of size $R$ scales as

$$I(s) \sim R^6$$
large particles can severely dominate the autocorrelation with the potential to hide the presence of smaller particles. In an attempt to obtain a clearer image of the particles present, AFM microscopy was performed.

5.2 Atomic Force Microscopy

An AFM forms a topological image of a region of interest of a material by scanning a needle-like structure, called a cantilever, with very sharp tip over the sample surface. As the tip of the cantilever approaches the surface of the region being scanned, Coulomb forces of attraction between the cantilever’s tip and the surface causes the cantilever to deflect towards the surface. As the tip of the cantilever touches the surface, increasing Coulomb force of repulsion causes the cantilever to deflect away from the surface. These deflections are picked up by a laser beam that is incident on the flat top of the cantilever and reflected off to a position-sensitive photodiode (PSPD). Therefore, as the tip of the cantilever is raised or lowered over the surface the deflections are recorded, through the reflected light beam, by the PSPD. By using a feedback loop to adjust the tip of the cantilever, the AFM develops a topographic image of the scanned surface.

To get an AFM image, 60 wt.% glucose solution cooked at 90 °C for 6 days is coated on a microscope slide, dried and then etched for 10 minutes in a O₂ plasma to get a clear image of the surface. From the result shown in Figure 5.1 (and Figure 5.2), there is a low density of the popcorns compared to a new set of particles, 30±5nm, seen by the AFM. Therefore, from the results observed, it was suspected that at least two categories of particles are made from the hydrothermal treatment of glucose solution: the popcorns and the 30 ± 5nm sized-particles.
Figure 5.1: AFM photograph of the surface of a heated glucose solution sample. The image shows three large particle inside the yellow circles, 500 nm in size, suspected to be popcorns.
5.3 Dialysis

Since AFM shows particles of 30 nm size, dialysis was done to better determine the source of the optical properties, (fluorescence and absorption) observed. A dialysis cassette with a cut-off of 20 KDa was used. The cellulose membrane from this dialysis cassette allows molecules smaller than roughly 6 to 8 nm to pass through it and locks in larger molecules. This way, molecular particles bigger than 8 nm can be isolated.

5.3 Dialysis

The Dialysis cassette shown in figure 5.3 is a 20 KDa dialysis cassette with a cellulose membrane. The content of the cassette was a 60 wt. % sugar solution, heated at 140 °C for 3 hrs. whose initial absorption and fluorescence data are plotted in Figure 5.4. The filled dialysis cassette was then put into a 1000 ml Pyrex beaker containing Millipure water. The fluorescence and absorption of the Millipure water was also obtained (illustrated in Figure 5.7). The beaker containing the dialysis cassette and the Millipore water was covered with an aluminum foil and gently stirred by a magnetic
stir bar for 48 hrs. Samples from the dialysis cassette and Millipore water in the beaker were taken and tested for fluorescence and absorbance. It was observed that the intensity of absorption and fluorescence in the dialysis cassette decreased (illustrated in Figure 5.5) while the Millipore water, which had no fluorescence or absorbance initially, developed appreciable fluorescence and absorbance (illustrated in Figure 5.8). The water in the beaker was then exchanged with fresh Millipure water and the cassette was allowed to exchange for another 48 hrs. At the end of the second 48 hrs., there was little or no absorption and fluorescence in either the water in the beaker or the contents of the dialysis cassette (Figures 5.8 and 5.6 shows this respectively).

This signifies that the most contributors to the fluorescence and absorbance observed in chapter four was due to the particle lesser than 8 nm in size. The popcorn and the 30 ± 5 nm particles contribute little or nothing to the optical properties.

Figure 5.3: Photograph of a 20kDa dialysis cassette used for the dialysis procedure.
Figure 5.4: Before the commencement of the dialysis procedure, the cooked sugar samples (in the dialysis cassette) were seen to have strong absorption and photoluminescence.
Figure 5.5: At the end of the second 48 hrs., it was observed that there was little or no photoluminescence and absorption in the content of the dialysis cassette.
Figure 5.6: At the end of the second 48 hrs., it was observed that there was little or no photoluminescence and absorption in the content of the dialysis cassette.
Figure 5.7: The Millipure water, used as an exchange medium, showed no absorption and photoluminescence before the start of the dialysis procedure.
5.3. Dialysis

Figure 5.8: After 48 hrs. of dialysis the Millipure water was seen to have strong photoluminescence and absorption. This is suggestive of the fact that particles lesser than 8 nm in size are the primary source of these optical properties.
Figure 5.9: At the end of the second 48 hrs., it was observed that there was little or no photoluminescence and absorption in the changed Millipure. This portrays that the majority of the absorption and photoluminescence sources were extracted in the first 48 hrs.
Chapter 6

Conclusion

From this study, it is observed that hydrothermally treated glucose solutions form large particles (popcorns) and display increasing absorption/fluorescence, that mirrors CNDTs in literature, with heating time. As seen form the dialysis results, most (if not all) of the absorption /fluorescence is not caused by the popcorn, but due to very small (< 8 nm) particles that could be merely due to molecular by-products of the caramelization process [12] or the carbon dots. It is not clearly verified if hydrothermally treating glucose solutions at different temperatures create well-defined carbon dots.

Future work would necessitate conducting series of dialysis to separate out the various particles by size and then investigate these particles individually using fluorescence microscopy to determine their properties. It is also important to test for wavelength-dependence emission by conducting photoluminescence spectroscopy for various excitation wavelength in order to ascertain if CNDTs are indeed produced by hydrothermally-treating sugar solutions.
Appendix A

Extinction Coefficients

The results for the extinction coefficients measurements done in chapter 3 are presented in this section. The extinction coefficient is the gradient of the line relating absorbance of the sample solution at various concentrations. It is a measure of how much the sample (cooked sugar solutions) decreases the intensity of light at a given wavelength. The aim of this section is to investigate how the extinction coefficient of cooked glucose solutions is affected by temperature, duration of cooking and concentration.

Extinction coefficients for solutions in general are measured in litre per mole per centimeter ($Lmol^{-1}cm^{-1}$). The units of measurement used in this investigation is per wt.% per centimeter ($wt.%^{-1}cm^{-1}$) since the unit of concentration used is the wt.%. Specifically, the extinction coefficients from the following plots are measured in units of $(I(0)wt.%)^{-1}$, where $I(0)$ is the starting concentration of the cooked sugar solution used in the procedure. (The new concentration is due to the dilution done in chapter 3 after cooking the sugar solutions).
Figure A.1: Extinction coefficient plot for the absorption at 280 nm from a 20 wt.% sample cooked at 80 °C for 12 hrs.

Figure A.2: Plot of absorption at various concentrations for a 20 wt.% sample cooked at 80 °C for 12 hrs.
Figure A.3: Extinction coefficient plot for the absorption at 230 nm from a 20 wt.% sample cooked at 80 °C for 48 hrs.

Figure A.4: Extinction coefficient plot for the absorption at 280 nm from a 20 wt.% sample cooked at 80 °C for 48 hrs.
Figure A.5: Plot of absorption at various concentrations for a 20 wt.% sample cooked at 80 °C for 48 hrs.

Figure A.6: Extinction coefficient plot for the absorption at 230 nm from a 20 wt.% sample cooked at 80 °C for 400 hrs.
Figure A.7: Extinction coefficient plot for the absorption at 280 nm from a 20 wt.% sample cooked at 80 °C for 400 hrs.

Figure A.8: Plot of absorption at various concentrations for a 20 wt.% sample cooked at 80 °C for 400 hrs.
Figure A.9: Extinction coefficient plot for the absorption at 230 nm from a 40 wt.% sample cooked at 80 °C for 12 hrs.

Figure A.10: Extinction coefficient plot for the absorption at 280 nm from a 40 wt.% sample cooked at 80 °C for 12 hrs.
Figure A.11: Plot of absorption at various concentrations for a 40 wt.% sample cooked at 80 °C for 12 hrs.

Figure A.12: Extinction coefficient plot for the absorption at 230 nm from a 40 wt.% sample cooked at 80 °C for 48 hrs.
Figure A.13: Extinction coefficient plot for the absorption at 280 nm from a 40 wt.% sample cooked at 80 °C for 48 hrs.

Figure A.14: Plot of absorption at various concentrations for a 40 wt.% sample cooked at 80 °C for 48 hrs.
Figure A.15: Extinction coefficient plot for the absorption at 230 nm from a 40 wt.% sample cooked at 80 °C for 400 hrs.

Figure A.16: Extinction coefficient plot for the absorption at 280 nm from a 40 wt.% sample cooked at 80 °C for 400 hrs.
Figure A.17: Plot of absorption at various concentrations for a 40 wt.% sample cooked at 80 °C for 400 hrs.

Figure A.18: Extinction coefficient plot for the absorption at 230 nm from a 60 wt.% sample cooked at 80 °C for 12 hrs.
Figure A.19: Extinction coefficient plot for the absorption at 280 nm from a 60 wt.% sample cooked at 80 °C for 12 hrs.

Figure A.20: Plot of absorption at various concentrations for a 60 wt.% sample cooked at 80 °C for 12 hrs.
Figure A.21: Extinction coefficient plot for the absorption at 230 nm from a 60 wt.% sample cooked at 80 °C for 48 hrs.

Figure A.22: Extinction coefficient plot for the absorption at 280 nm from a 60 wt.% sample cooked at 80 °C for 48 hrs.
Figure A.23: Plot of absorption at various concentrations for a 60 wt.% sample cooked at 80 °C for 48 hrs.

Figure A.24: Extinction coefficient plot for the absorption at 230 nm from a 60 wt.% sample cooked at 80 °C for 400 hrs.
Figure A.25: Extinction coefficient plot for the absorption at 280 nm from a 60 wt.% sample cooked at 80 °C for 400 hrs.

Figure A.26: Plot of absorption at various concentrations for a 60 wt.% sample cooked at 80 C for 400 hrs.
For different concentrations, the value of the extinction coefficients (related to the gradients in the plots) increased with cooking time. It was also observed that samples for the same time duration at higher temperatures had higher extinction coefficient values. From the 60 wt.% and 40 wt.% plots, we observe that the extinction coefficient for the absorption of the 230 nm wavelength starts out relatively higher than that of the absorption at 280 nm. With further heating, the value of the extinction coefficients increases relatively faster for absorption at the longer wavelength.
Appendix B

Plots

B.1 PCS Plots

Figure B.1: PCS plot of 20 wt.% cooked at 140 °C.

Figure B.1: PCS plot of 20 wt.% cooked at 140 °C.
B.2. Photoluminescence Plots

Figure B.2: PCS plot of 60 wt.% cooked at 80 °C.

Figure B.3: Photoluminescence plot of 20 wt.% cooked at 100 °C.
B.2. Photoluminescence Plots

Figure B.4: Photoluminescence plot of 20 wt.% sugar solution cooked at 140 °C.

Figure B.5: Photoluminescence plot of 40 wt.% glucose solution cooked at 80 °C.
B.2. Photoluminescence Plots

Figure B.6: Photoluminescence plot of 60 wt.% glucose solution cooked at 80 °C.

Figure B.7: Photoluminescence plot of 60 wt.% glucose solution cooked at 100 °C.
Figure B.8: Photoluminescence plot of 60 wt.% glucose solution cooked at 120 °C.

B.3 Absorption Plots

Figure B.9: Absorption plot of 20 wt.% glucose solution cooked at 80 °C.
B.3. Absorption Plots

Figure B.10: Absorption plot of 60 wt.% glucose solution cooked at 80 °C.

Figure B.11: Absorption plot of 40 wt.% glucose solution cooked at 100 °C.
B.3. Absorption Plots

Figure B.12: Absorption plot of 40 wt.% glucose solution cooked at 120 °C.

Figure B.13: Absorption plot of 60 wt.% glucose solution cooked at 120 °C.
B.3. Absorption Plots

Figure B.14: Absorption plot of 20 wt.% glucose solution cooked at 140 °C.

Figure B.15: Absorption plot of 60 wt.% glucose solution cooked at 140 °C.
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