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PUTRESCINE CATABOLISM WITH CUCY AS A NEW FORM OF CANCER THERAPY

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PUTRESCINE CATABOLISM WITH CUCY AS A NEW FORM OF CANCER THERAPY

by

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ABSTRACT

PUTRESCINE CATABOLISM WITH

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CANCER THERAPY

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Faculty Mentor: Wei Chen

There are several types of cancer treatments, but, unfortunately, many types also target healthy, non-tumorous cells. This possible treatment therapy would only target cancerous cells with the use of the nanoparticle Copper Cysteamine (CuCy) and its effects on the polyamine known as putrescine. Putrescine is a positively charged cation that plays an important role in balancing out negatively charged components of our cells, such as DNA, RNA, and proteins. Though usually tightly controlled by feedback mechanisms, putrescine in cancerous cells is at unnaturally high levels. This experiment looks at using CuCy to lower the levels of putrescine in a cell as a possible new form of cancer treatment. Variables such as pH, varying concentrations, temperature changes, etc. will also be observed. Changes will be measured via absorbance peaks from a UV-Vis spectrometer and using Fourier-transform infrared spectroscopy. CuCy was found to be a possible

influential factor in controlling putrescine levels in certain environments. Future studies could test this relationship with other polyamines that are naturally present in the body and further establish this relationship with cell study.

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CHAPTER 1

INTRODUCTION

Cancer research has been widely studied in an effort to find treatment options that are effective, inexpensive, and do not bring harm to the patient. The latter often happens in cancer treatment when tumor cells are being treated. Unfortunately, many forms of treatment also target healthy, non-tumorous cells. On top of that, there are treatment methods that still do not effectively decrease growth in certain forms of cancer. This brings reason for other types of cancer treatment to be studied and expanded upon. A promising new method of cancer therapy involves use of the luminescent, copper-based nanoparticle that is known as Copper Cysteamine (CuCy). Another angle that cancer therapy has been approached from has included the control of polyamines, like putrescine, within cells. The relationship between CuCy and putrescine may lead to a new, amplified form of cancer therapy. The combination of these two would lead to not only a significant decrease in tumor growth, if not stopping growth all together, but also more focused treatment in localized regions. This, as previously mentioned, is essential for making sure healthy cells are not targeted.

1.1 Polyamines and Putrescine

Polyamines are positively charged cations that are naturally found in cell. They play an important role in balancing out negatively charged components of our cells, such as DNA, RNA, and several proteins that we make. They are also used in a number of

cellular processes involving growth and maintenance of our body's cells (Minois et al., 2011). As many biological components in our body, polyamines are regulated through a variety of feedback mechanisms that keep its levels well controlled. However, studies show that this is not the case in cancerous tissue. In fact, high polyamine levels have been linked to breast cancer, skin cancer, prostate cancer, lung cancer, etc. (Nowotarski et. al, 2013). As mentioned, polyamines are often an essential factor in cell growth regulation, so this correlation is not unexpected. In fact, this link has opened many doors in research within cancer therapy.

There's been success at using polyamines as tools to diagnose cancer early on in patients when looking at urine or plasma samples (Park and Igarashi, 2013). Another important avenue in cancer research with polyamines has been studies that further look into the dynamic interactions between these positively charged complexes and several pathways that drive cancer, such as PTEN-PI3K-mTOR complex 1 and WNT and RAS signaling pathways (Casero et al, 2019). These impacted pathways show the complexity and diverse roles of polyamines in the cell. PTEN-PI3K-mTOR complex 1, for example, regulates polyamine metabolism (Zabala-Letona et al, 2017). Pathways like WNT target polyamines by normal regulation and also via renewal of cancerous cells (Li et al, 2020). The RAS signaling pathway was studied to be involved in general proliferation of cells (Bachrach et. al, 2001).

The fact that polyamines have so many important roles in several of these pathways show how effective a cancer treatment would be that could control the levels of polyamines in a cancer cell. This would influence several different pathways and reactions that allow

cancers to grow, effectively putting a hold on growth, and depending on the pathway that's being affected, it could significantly reduce cancerous tumor growth.

In order to narrow down the scope of the experiment, as well as keep consistent results, the polyamine known as putrescine was used. Putrescine is a diamine that has four carbons and is often produced during tissue decomposition. More specifically, putrescine is produced during decarboxylation of arginine and ornithine (National Center for Biotechnology Information, 2020). When looking into precursors to lung cancer, it was found that a rapid uptake of putrescine was present. This accumulation was seen in almost every cell that was associated with tumor growth (Hoet et. al, 1996). Another study found that in samples studying brain cancer, putrescine levels were almost the same across the board on normal cells, but were not only greater in cancerous tissues cells, but proportionally greater as per tumor size (Harik & Sutton, 1979). Cells in the human body are sensitive to changes in putrescine levels, so figuring out a way to monitor and manipulate how minor changes effect cancer growth will shed light on developing new forms of cancer therapy.

1.2 Nanoparticles and CuCy

Nanoparticles, as the name suggests, are small in nature - under 100 nm to be precise. They've been invaluable in modern medicine due to their variable applications as tracing components in imaging, carriers in drug therapy, gene delivery, and tumor suppression treatment (Murthy, 2007). With the wide range of nanoparticles that have been developed, along with their ability to often withstand a number of extreme conditions, their worth is rather limitless.

There are also practical considerations in drug delivery when it comes to nanoparticles. In this experiment, this is in the form of polyethylene glycol (PEG)-functionalized nanoparticles. This simply denotes that a coating essentially will be around the nanoparticles that aid in protecting the nanoparticle from phagocytosis, opsonization, etc. Furthermore, the PEG functionalization also results in better circulation in the cell and decreasing chances of being targeting by the body's immune system (Suk et. al, 2016). All in all, this is a great way to improve drug success when it comes to treatment in more practical settings within cells.

The nanoparticle used in this experiment is CuCy. CuCy is a photosensitizer as well as a radiosensitizer that is activated by light, X-ray exposure, and various other types of waves. Its potential as a form of cancer treatment has been tested on colorectal cancer cells and resulted in apoptosis and autophagy. Though the mechanism of this nanoparticle decreasing tumor growth isn't completely understood, it has been seen that there is significant change to the mitochondrial membrane potential of a cell when using this nanoparticle (Liu et. al, 2017). There are several links here that can relate to polyamines like putrescine, and hopefully this shows an amplified effect in stopping the growth of cancerous cells.

1.3 Putrescine and CuCy

Seeing as how the mitochondrial membrane potential relies heavily on charged components, the effect of CuCy could have a great effect on decreasing the intake of the cationic putrescine. This research would allow a noninvasive form of cancer therapy that could also leave healthy cells untouched. Because this nanoparticle has the ability to be activated when needed or in a particular area, there's much control in this treatment

therapy. There would be an extremely beneficial form of cancer therapy if CuCy could decrease putrescine levels in a cell, and this could further shed light on the possible mechanism in which CuCy works under. Therefore, the overall goal of this study is to understand the relationship between putrescine and CuCy under a number of different conditions

CHAPTER 2

METHODOLOGY

2.1 PEG-Functionalized CuCy Preparation

To begin, 273 mg of Copper Chloride was dissolved in 50mL of room temperature DI H20. Then, 370 mg of Cysteamine was mixed into the solution, along with 40 mg of PEG. This entire mixture was heated at 100 degrees Celsius for about 10 minutes. After this, the pH of the solution was brought to 7 using the necessary amount of 2M NaOH. There was also a visual indicator of the solution turning a clear, golden color at this point. The mixture continued to stay on the heat and was continuously stirred for 10 minutes. It was taken off the heat when the solution changed from a clear golden color to a cloudy, white to purple color (Chudal et. al, 2020; Kazempour et. al, 2019).

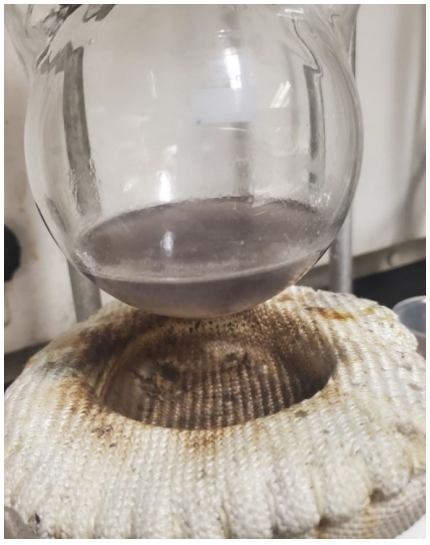

Figure 2.1: PEG-Functionalized CuCy Solution. Prepared solution that should be taken off heat at this point to follow a washing and drying process PEG-Functionalized CuCy

Figure 2.2: PEG-Functionalized CuCy Solution with Ultraviolet Light. Using this flashlight, the luminescent, PEG functionalized CuCy nanoparticles are now visible.

The mixture is allowed to cool and then centrifuged. The unnecessary portion was discarded while the nanoparticles that were left were then washed with ethanol and DI water. This process of centrifugation and washing was done for a total of three times. The remaining product is then vacuumed into a powder overnight at room temperature (Chudal et. al, 2020).

The powder form of PEG-functionalized CuCy was collected and stored into an airtight container. 1.6 mg was measured and dissolved in 0.5 L of DI water. Using, this solution in combination with 1.8 uM solution of putrescine, tests were run in a Fourier Transform Infrared Spectrometer (FTIR).

2.2 FTIR Samples

First, putrescine was run through the FTIR machine in liquid form and the graph data was collected. Then, a mixture of equal amount of putrescine and PEG-functionalized CuCy was mixed together, and this liquid sample was run as well. The graph of this run was collected.

2.3 UV-Vis Spectrophotometer Samples

Cuvettes of samples were loaded into the UV-vis spectrophotometer. The first sample contained pure PEG-functionalized CuCy. The next test was done using just putrescine while the following contained 1 ml of each solution. Gradually, more water was added, 1 ml at a time, until the cuvette was full. Each of these runs were measured and then run again after allowing the sample to sit for 1 hour. All of this data was collected and graphed.

The next set of runs that were collected involved using citric acid. Similarly done with the dilution tests, 1 ml of citric acid was continuously added between runs until the cuvette was full. Also, the same trend of waiting 1 hour from when the sample was initially run took place as well. All the data was collected and graphed. In addition, pH readings were collected between runs of each sample as well.

2.4 Other Tests using pH

Some other tests were conducted that tested how the interaction between PEG-functionalized CuCy and putrescine took place under varying concentrations. The measurement was taken using both pH and a visual indicator of luminance in each of the samples. The samples mixed and measured included: pure putrescine, pure PEG-

functionalized CuCy, ratios of 1:2 or 2:1 of putrescine and PEG-functionalized CuCy mixed together.

CHAPTER 3

RESULTS

3.1 FTIR Results

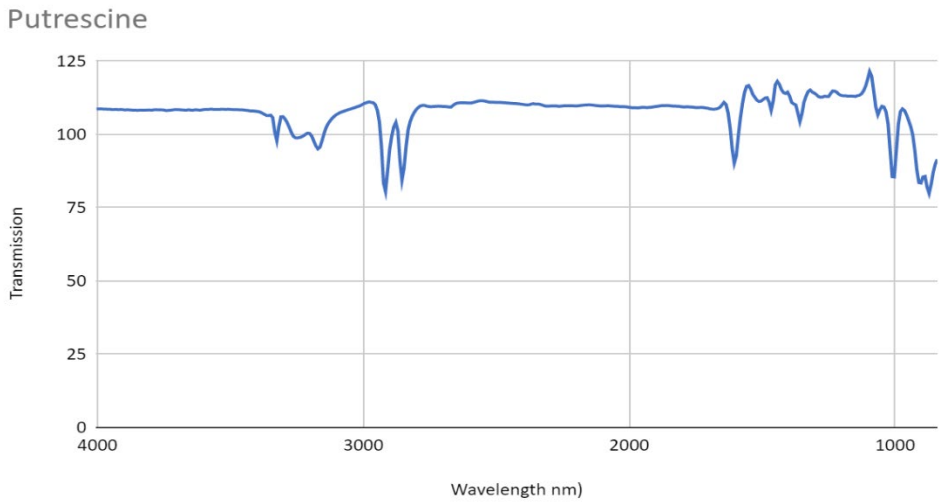

Figure 3.1: FTIR run of Putrescine

The initial run of just putrescine was taken and confirmed as a reliable, non-contaminated reading by noting that key peaks that were supposed to be present were at the correct wavelengths. There was a double peak just below 3000 nm, and there is a smaller double peak around 3200 nm as well (Figure 3).

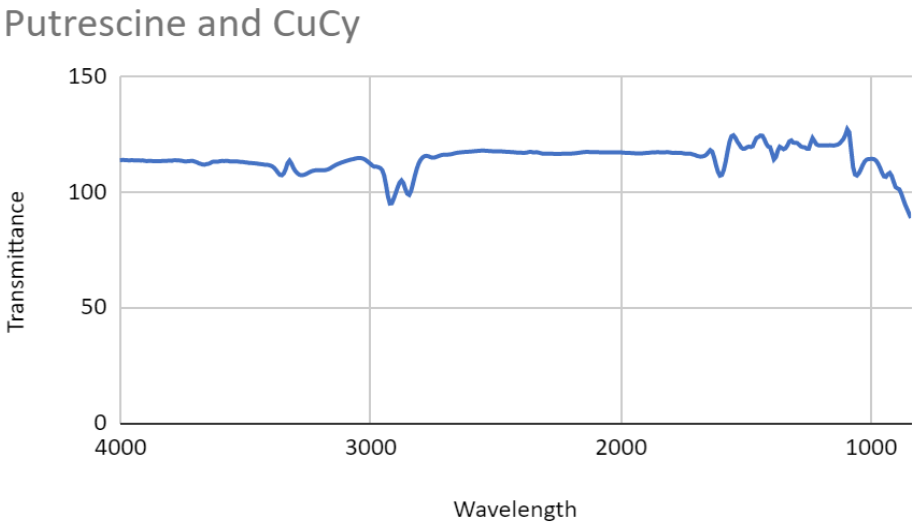

Figure 3.2: FTIR Run of both Putrescine and CuCy

The next run was as sample of PEG-functionalized CuCy and putrescine. This was taken and compared to the initial run that was of just putrescine. The double peak just below 3000 nm reduced in size significantly, and there is a smaller double peak that was around 3200 nm is nonexistent (Figure 3 and Figure 4).

3.2 UV-Vis Spectrophotometer Results

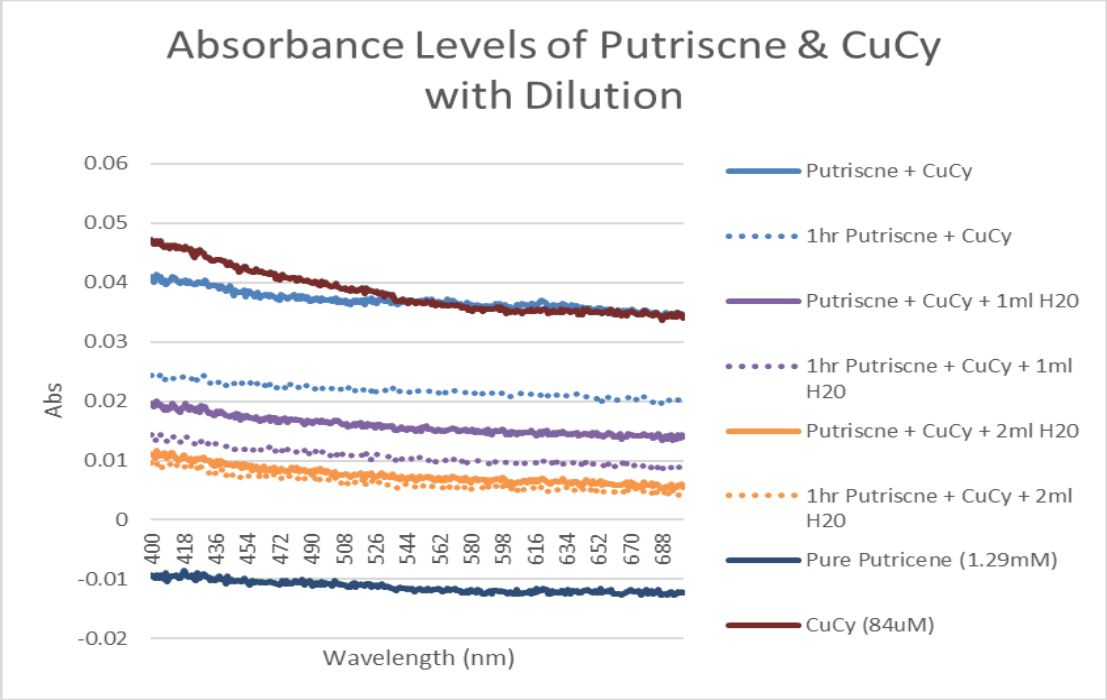

Figure 3.3: UV-Vis Spectrophotometer Runs of Putrescine and CuCy with Dilution. These runs of putrescine and CuCy with controls and varying dilution levels. The dotted lines represent measurements taken after 1 hour.

The UV-Vis Spectrophotometer runs with dilution showed a consistent degradation of CuCy. This trend is also seen with the dotted lines that show the same samples 1 hour later (Figure 5).

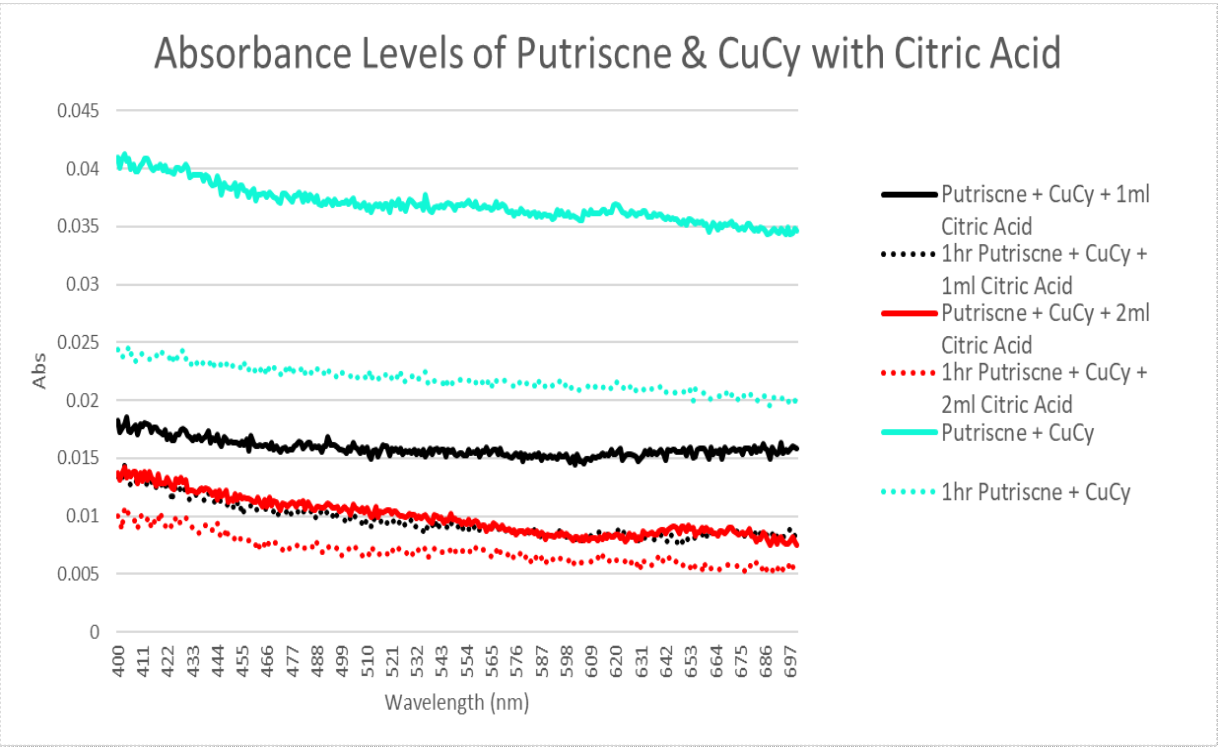

Figure 3.4: UV-Vis Spectrophotometer Runs of Putrescine and CuCy with Acid. These runs of putrescine and CuCy with varying acidity levels. The dotted lines represent measurements taken after 1 hour.

The UV-Vis Spectrophotometer runs with an acidic component shows, once again, a consistent degradation of CuCy. This trend is still seen with the dotted lines that show the same samples 1 hour later (Figure 5).

3.3 pH Results

Table 3.1: pH readings with putrescine, CuCy, and Citric Acid. This table represents pH measurements that were taken with varying samples amount of putrescine, CuCy, and Citric Acid

Sample	pH
Putrescine	10.44
CuCy	5.67
1:1 Putrescine and CuCy	9.90
2:1 Putrescine and CuCy	10.2
1:2 Putrescine and CuCy	8.40
1:1:1 Putrescine and CuCy and Citric Acid	4.95
1:1:2 Putrescine and CuCy and Citric Acid	2.99

Putrescine, which is more basic by nature, counter acts the more acidic CuCy. The pH readings between these two were found to be between the pH readings on each individually. In the samples with citric acid as a component, the pH understandably goes down, reaching readings lower than any combination of CuCy and putrescine that was obtained.

CHAPTER 4

DISCUSSION

Cancer Therapy is a wide and diverse field that encompasses so many forms of treatment, including Chemotherapy, surgery, radiation therapy, immune therapy, hormones therapy, stem cell transplants, etc. (U.S. Department of Health and Human Services, 2020). In many of these treatment forms, important factors such as cost, harm to healthy cells, and overall efficiency has led to continuous development in this field of study. In this study, a form of radiation therapy using nanoparticles by targeting compounds known as polyamines.

Specifically, this experiment has looked into the nanoparticle CuCy and the polyamine putrescine. There were some components that need to be considered when working with these compounds. Firstly, putrescine is rather volatile, so the quickly evaporative state needs to be considered when running these studies. Also, CuCy has a natural tendency to settle quickly, so sonication of the stock solution needs to be conducted before every run.

4.1 Analyzing FTIR Results

To begin, FTIR experiments were conducted. These tests were done in order to get understand the relationship between infrared intensity and wavelength of light in each of our samples. This was used because this relationship gives insight to the kinds of functional groups that are found in samples. This is done despite effects of differing temperature, pressure, etc. (Smith, 1996).

Also, it's important to note that these samples absorb radiation of very particular wavelengths, which leads to changes in dipole moments within the sample. The reason this is emphasized is because this change in a dipole moment can relate to changes in energy levels and transitions (Birkner & Wang, 2020). It's a deeper understanding as to what kind of molecule is being obtained from these samples when different components are mixed together.

As briefly mentioned, particulars components stand out int FTIR graphs, such as functional groups. Putrescine is also known as butane-1,4-diamine. As seen in the name, this compound has several C-H bonds and 2 amino groups (Figure 7).

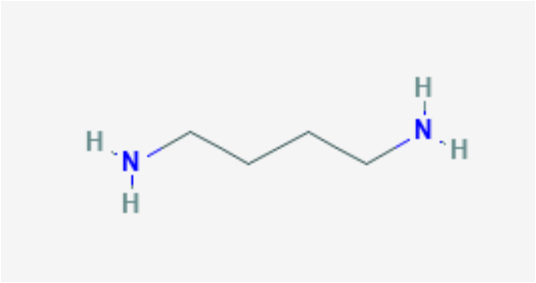

Figure 4.1 Chemical Composition of Putrescine (Image from the National Center for Biotechnology Information, 2021).

The C-H double peak stretch and amines are seen at just below 3000 nm and around 3200 nm, respectively (Figure 3). This is important to keep note of as the run combined with CuCy is analyzed. It's interesting to see that with the addition of CuCy, these parts of the graph change. There is a reduced intensity at the C-H stretch and almost a nonexistent peak at where the amines were once accounted for (Figure 4). This would mean that CuCy has likely resulted in created some kind of degradation when it comes to putrescine. However, further testing should be conducted on this matter in order to further verify these results.

4.2 Analyzing UV-Vis Spectrophotometer Results

For the UV-Vis Spectrophotometer, the absorbency of different samples were measured. Specifically, by using this method, the absorbance of energy in the form of light or electromagnetic radiation is measured. Therefore, the samples measured are excited from the ground state to the singlet in that particular material being analyzed (Raja & Barron, 2021). For the dilution measurements, the samples were analyzed 1 hour after the initial measurement in order to see if further degradation took place. This is important because the addition of water will naturally bring down absorbency, but the additional wait time ensured that this change is not simply due to the water. Naturally, there was a consistent trend when looking at dilution, but, more importantly, this trend was also seen within the 1-hour lapse (Figure 5). This result shows that the decrease in absorbency that took place was able to be partially attributed to the reaction that occurred between putrescine and CuCy.

It's also noteworthy that this only applies to the degradation and analysis of CuCy. Putrescine does not efficiently absorb light, as denoted by the extremely low absorbency level recorded (Figure 5). Even if there was a shift chemically, it would be difficult to determine with this analytical method for putrescine. Therefore, these results essentially show how CuCy is impacted in the presence of putrescine.

In the next graphs that are shown, citric acid was used due to CuCy having shown to have a preference in acidic conditions (Chudal et. al, 2020). Specifically, the acid that was used was citric acid. Citric acid is naturally found in the body and works quite well in areas of inflammation, peroxidation, DNA fragmentation, etc. (Abdel-Salam et. al, 2014). Hopefully, this can be applied to more practical testing in future studies. For the tests run,

these results show a downward trend in absorbency levels with the further addition of acid (Figure 6). This continues to represent that there is a continuous degradation of CuCy in the presence of putrescine in both acidic and non-acidic environments.

4.3 pH Analysis

The reading of putrescine came out to be 10.44 on the pH, denotating quite a basic nature. This counter acts the more acidic nature CuCy, which sits close to a pH of 6 (Table 1). It make sense that the mixing of these two components results in a pH that is in the middle of these two readings. Though the pH is still far from a pH that would be ideal in the human body. Also, as previously stated, CuCy has showed to have an acidic preference when it comes to environmental efficiency. This is a factor that be manipulated by changing the concentration, the addition of acids/bases, etc. As for the citric acid component, the pH does indeed go quite low. This is expected and, again, could be an important factor for creating ideal pH reliant environments when it comes to future testing.

4.4 Future Studies

Future stages of this experiment can look into how this relationship does in the presence of important factors in cancer treatment such as reactive oxygen species (ROS). ROS species are important to regulate in the body because elevated levels can lead to gene damage, cellular pathway disruption, and even some pathological conditions (Perillo et. al, 2020). Other tests include conducting cellular assays that would be used to see how the relationship between CuCy and putrescine plays out in actual cells. This would be done in both cancerous and non-cancerous cells in order to compare the decrease in growth and if the would be a potentially harmful effect to healthy cells of the human body. Of course,

there are a number of variables to be considered here, but the ideal end result would be sole targeting of cancerous cells.

All in all, hopefully future studies on this matter can closely monitor putrescine with CuCy in such a way that opens the doors to a new form of cancer therapy.

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BIOGRAPHICAL INFORMATION

Prishmi Nagarajan's interest in science began at a young age. With hope of serving as a doctor one day, she decided to continue her academic journey at UTA. Knowing she wanted to conduct research in a field that would contribute to medical treatment, she gravitated towards the lab that she's overjoyed to be giving the opportunity to work in. Dr. Wei Chen and graduate students like Eric Amador, Nil Pandey, and Christina Xing were always willing give her direction and advice. She feels fortunate to have had such a positive research experience in her first lab and hope to continue volunteering after she graduates to continue her research in cancer therapy.

She also hopes to go to medical school and one day become a pediatrician or an ophthalmologist. Along with doing so, she would still like to continue her research with nanoparticles for cancer therapy treatments. As for the near future, she'll be soon graduating with a B.S in Biology from UTA and be off to work in a hospital while continuing her research.