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The Role of Ionic Liquids in Gold Nanorod Synthesis

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ABSTRACT

The term cancer is used to define a large set of medical conditions that result from uncontrolled cell proliferation. The severity of cancer ranges depending on several factors, but it is one of the leading causes of death in approximately 60% of the world. Current treatment options are often not efficacious and face many limitations especially regarding limiting the secondary damage to healthy cells. Gold nanorods have shown to be a potential solution for effectively overcoming many of these barriers, but their employment is limited due to toxicity concerns stemming from the use of hexadecyltrimethylammonium bromide (CTAB) in their synthesis. This research has thus aimed to develop a methodology for synthesizing gold nanorods that utilizes ionic liquids in replacement of CTAB. This was attempted by following standard seed-mediated methods in which first gold salt is reduced and combined with the surfactant CTAB to create a seed solution, then a growth solution is created with silver to promote deposition, and finally the combination promotes growth. The end goal would allow for the synthesis of gold-nanorods whose size and aspect ratios are able to be manipulated for use in biological systems.

Through this project, it was shown the size of the nanostructure produced was dependent on many variables. The identity of the ionic liquid, the amount of silver ions, and the potential for competing reduction pathways all proved to be significant challenges towards controlling the nanostructure produced. Unfortunately, UV-Vis spectroscopy indicated the production of spherical geometries over the rod-like geometries that were intended. While the research was not successful, it provided insight towards the mechanisms that could lead towards successful experimentation in the future.

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LIST OF ABBREVIATIONS

CTAB	Hexadecyltrimethylammonium Bromide
IL	Ionic Liquid
LSPR	Localized Surface Plasmon Resonance
NIR	Near-infrared Radiation
NP	Nanoparticle
PDI	Polydispersion Index
PPTT	Plasmonic Photothermal Therapy
UV-Vis	Ultraviolet-Visible

CHAPTER 1: INTRODUCTION

Cancer and the Lethality of Metastasis

Cancer is the broad term used to describe a set of medical conditions that result from the uncontrolled proliferation of one or more kinds of cells within the body. This proliferation eventually forms masses within the body known as tumors. Tumors may be either benign, localized and noninvasive to other tissue, or malignant, able to spread and invasive to other tissues (1). While there are over a hundred different types of cancer, they can be classified within categories based on the type of cell affected. These categories include classifications such as carcinomas, sarcomas, leukemias, and lymphomas (1).

The development of cancer is a multistep process that is influenced by numerous intertwining factors. In general, the development of cancerous cells and tumors arises when a once normally functioning cell has incurred a series of alterations that disrupts its regulatory processes (1). For example, tumor initiation can result from factors such as the activation of an oncogene or DNA methylation of cell cycle regulators (3). This damage results in the cells being termed cancerous and causing the cells to deviate from their normal behaviors and processes. Two of these changes are especially important in the understanding of cancer lethality. The extracellular matrix of the tissue is a network of collagen, proteoglycans, laminin, and fibronectin that surrounds cells and provides functions such as structural support, cell adhesion, and cell to cell communication (4). Cancerous cells can secrete proteases that decompose this matrix and weaken its integrity. They can also secrete a variety of growth factors. One role of these growth factors includes the formation of new capillaries towards the cancerous cell. The new capillaries then provide the tumor with the oxygen and nutrients it needs to continue

growing. More importantly, these new capillaries allow the cell to enter the bloodstream and undergo metastasis (1).

The lethality of cancer varies widely and is subject to many complex factors. However, it is between the first two leading causes of death before age seventy in approximately 60% of the world (2). Of all cancer deaths, over 90% of them can be attributed towards metastasis (5). During metastasis the cancerous cells travel to secondary locations and form additional tumors. After this stage, the probability of being able to fully eradicate the cancerous cells is often impossible (6). While many current treatments for cancer exist, most exhibit barriers that prevent them from success. One of the main problems these treatments face is finding ways to eliminate cancerous cells while minimizing secondary damage to healthy cells. Especially in the case of solid tumors, treatments often may lead to toxicity with low rates of efficacy (1). They also face many obstacles biologically within the formats of the drug delivery systems used (10). These problems emphasize the importance of preventing cancerous cells from undergoing metastasis, but previous attempts to develop drugs that prevent metastasis have not been efficacious (5). Therefore, more research is needed to develop novel methods that can overcome the current limitations.

Ionic Liquids in Healthcare

Ionic Liquids (ILs) are substances that consist completely of ions held together by electrostatic forces and are liquid across a broad range including room temperature. One would expect that the strong ionic forces within ILs would normally cause associations or formations into crystal lattices. However, the cations are composed of molecules that are asymmetric and sterically hindered, resulting in their ability to overcome this (8). Due to such, they display

unique properties such as having high viscosities, high electrochemical stability, and low partial pressures. They also present compatibility with a large range of different possible cations and anions allowing for tunability within the choice of chemical properties. This has resulted in their popularity in a variety of fields such as in batteries and solar cells (7).

Recently, the potential applications of ILs in healthcare has gained popularity, including in drug delivery. ILs have been shown to improve the performance of a variety of pharmaceutical components by modifying drug solubility and increasing thermal stability (9). They have also been reported to display antitumor, antiviral, and antibacterial properties (11). Of particular interest is the increased ability for their use within transdermal delivery systems. ILs have shown the ability to breach the stratum corneum that usually exists as a barrier for similar systems (8). However, the possibility of ILs being used in delivery systems raises concerns about their toxicity. Previous research has confirmed the toxicity of many ILs created, but it has also confirmed that the toxicity is tunable. Depending on the factors such as choice of cation, anion, or substituent, the toxicity can be lowered. Interestingly, ILs with cholinium-based cations exhibit much lower levels of toxicity than most of their counterparts and produce less skin irritation (8). Even though the current research stresses the continual development of improving these processes, one can see the usefulness of employing ILs especially to that of transdermal delivery. Due to their lower toxicity, cholinium-based ILs particularly show particular interest for the purposes of this thesis.

Gold Nanorods Healthcare

Nanotechnology refers to the field that designs and applies materials and devices that have functional units on the nanoscale (12). Nanotechnology has gained a lot of popularity

within recent years due to its wide range of applications within areas like construction, food processing, and agriculture (13, 14). The use of nanoparticles (NPs) within medicine shows promise at being able to revolutionize medical treatments (15). The size and surface area to volume ratio of NPs distinguishes them from bulk material, resulting in changes in their chemical, physical, and optical properties. (16, 17). As a result, they have been used in diagnostic, analytic, and therapeutic contexts (16) including in cancer therapy and enhancing drug delivery systems (17, 18).

NPs can be sorted into broad classes dependent on their morphology or chemical properties including ceramic, polymeric, metal NPs, and more (17). The research of this thesis is particularly interested in the development of gold metal NPs. Gold NPs display properties that are distinct from their bulk counterpart. The conduction electrons of gold NPs can be excited by the electromagnetic fields of light radiation, and, when struck, they oscillate collectively at the same frequency of that light. This phenomenon is known as localized surface plasmon resonance (LSPR) (19, 22). A portion of this light is re-radiated through scattering, but the majority is absorbed and converted to localized heat to achieve nonradiative relaxation (19, 20). The position and properties of this plasmon band can be altered depending on the size, shape, and chemical environment of the gold nanorod. In comparison to other gold nanostructures, gold nanorods are unique in that they can be tuned to absorb near-infrared radiation (NIR) more strongly and heat localized areas more effectively (21). This has caused gold nanorods to be investigated for their use as a cancer therapy option via plasmonic photothermal therapy (PPTT). In PPTT, gold nanorods absorb NIR light to generate localized heat and induce either tumor tissue apoptosis or necrosis (5, 21, 22). It is theorized that this technique would enhance positive

outcomes such as faster recovery times and result in fewer complications (20). However, complications such as the toxicity of gold nanorods act as barriers towards their implementation.

Gold Nanorod Synthesis Limitations

Despite the promising outlook of gold nanorod PPTT, gold nanorods still face uncertainty in their implementation due to concerns rooted within their synthesis. One challenge faced is the difficulty in achieving control over the determination of the resultant gold nanostructure's size and shape. However, it is a necessity due to the optical and catalytic properties of gold nanostructures being size-dependent (26). The wavelength of absorbance for the LSPR band as well as the plasmonic properties of gold nanorods is of great importance to therapies such as PPTT, and these aspects highly depend on the aspect ratio of the nanostructure (27, 28). This has resulted in a variety of methods being created to achieve mastery over these conditions. Seed-mediated growth is the most popular method for gold nanorod synthesis (19, 23). Within this method, the growth mechanism of the gold nanorods is not completely understood, and there are several suggested possible mechanisms (19, 24, 25, 26). The uncertainty arises from the complex interacting and synergistic set of thermodynamic, kinetic, and geometric factors that guides the structural development (28). For example, the geometric evolution within this method is primarily governed by whether the kinetic controls promote fast or slow nanocrystal development. The determination of the pathway leads to the formation of either high-index or low-index faceting nanoparticles, respectively. One aspect that guides this kinetic determination is the underpotential deposition of a foreign metal ion (28). This role is usually achieved by silver, but there are currently at least three differing mechanisms of how this is achieved (29). The most supported mechanism and often used method involves the role of the halide-containing

cationic surfactant hexadecyltrimethylammonium bromide (CTAB) (28). In this mechanism, CTAB forms rod-like micelle structures at concentrations above its critical micelle concentration (29). This surfactant then directs the shape in conjunction with silver by promoting bilayer growth on the nanorod faces and freeing the ends for elongation (24, 27, 28). A final factor of this synthesis involves the use of a reducing agent to reduce the gold metal salts (24). The problem with this method exists in that gold nanorods coated with CTAB have shown to be highly toxic and can cause damage to biological cells and tissues (23, 27). Removing CTAB from the solution also causes particle aggregation (27). This presents a problem with the current method as it traditionally uses high concentrations of CTAB (23). Developing a method for gold nanorods synthesis that would avoid these issues of toxicity could thus highly increase its applicability to biomedical systems.

CHAPTER 2: EXPERIMENTAL METHODS

Materials

Tetrachloroauric(iii) trihydrate, 99%; silver nitrate, 99%; sodium borohydride, 98%; L-ascorbic acid, 99% and choline bicarbonate, 80% were all purchased from Sigma Aldrich.

Methods

Ionic Liquid Synthesis

Ionic liquid synthesis. For all ionic liquids used within this thesis, choline bicarbonate served as the cation with carboxylic acids of variable alkyl chain lengths as the anions. An oil bath was set on a hotplate at 40 °C containing a round-bottom flask with a stir bar secured by a clamp stand. Following this, the necessary carboxylic acid was massed according to the desired ion ratio modifier and added to the round-bottom. Choline bicarbonate is then added slowly in 1 milliliter aliquots with a glass Pasteur pipette until the calculated quantity is obtained. Milli-Q water is then used to wash the transfer beaker before being added into the round-bottom. The reaction is then to remain at 40 °C overnight with continual stirring. The following day, the flask is transferred from the hot oil bath to the rotary evaporator. Before use, the hot water bath of the rotary evaporator is heated to 60 °C and the vacuum is set to a starting pressure of 500 millibar. After the addition of the IL, the pressure of the rotary evaporator is slowly decreased from 500 millibar to 15 millibar in small increments and sits for 1 hour. Following this, the dried IL is transferred to a scintillation vial and placed inside a vacuum oven at 60 °C for 48 hours. Before use, the purity of the IL was verified through ^{1}H nuclear magnetic resonance spectroscopy, and the water content was determined through Karl Fisher titration.

Gold Nanorod Seed-Mediated Growth

Preparation of the seed solution. To prepare the seed solution, a stir bar was added to a 20 mL scintillation vial and set on a magnetic stirring plate. To this vial, 25 microliters of 100 mM $HAuCl_4$ was added and mixed with 5 milliliters of 10 mL IL solution. 1 mL of 10 mM $NaBH_4$ was then added to the solution and stirred under vigorous conditions for 2 minutes. The solution was aged for 30 mins prior to use.

Preparation of the growth solution. The growth solution was prepared in 4 separate 20 mL scintillation vials with stir bars on a magnetic stirring plate. 5 mL of 10 mM IL solution was then added. All IL solutions used in the growth solution for this experimentation contained the same cation and anion as well as the same ion ratio as the previously prepared seed solution. Stirring was stopped, and variable volumes of a 40 mM $AgNO_3$ solution were added to each vial as desired. The solution was kept undisturbed for 2 minutes before the addition of 25 microliters of 100 mM $HAuCl_4$. Stirring was then restarted for the addition of 20 microliters of 64 mM ascorbic acid solution to each vial. Finally, 10 microliters of the seed solution were added to each of the growth solutions. The solutions were left for several hours with stirring before characterization.

Gold Nanoparticle Size and Charge Analysis. The size and zeta potential of the resultant gold nanoparticles was determined using the Malvern Zetasizer Pro Blue. To determine the size, 100 microliters of the gold nanoparticle solution was diluted with 900 microliters of Milli-Q water. The diluted solution was then transferred to a cuvette for analysis. The instrument parameters were set to account for the colloidal gold's refraction index of 0.18 and molar absorption of

3.433. The solution was then transferred from the cuvette to a zeta cell, and the zeta potential of the solution was determined. This process was then repeated for the seed solution.

Ultraviolet-Visible Spectroscopy Analysis. The UV-Vis spectrum of the resultant gold nanoparticles was determined using the UV-Vis spectrophotometer. Before analysis of the gold nanoparticles, the background was collected using Milli-Q water in a quartz cuvette. 100 microliters of the gold nanoparticle solution were diluted with 900 microliters of Milli-Q water and transferred to a quartz cuvette. This process was then repeated for the seed solution.

CHAPTER 3: RESULTS & DISCUSSION

Ionic Liquid Synthesis

The first step of the process was to synthesize ionic liquids that could serve as candidates to replace CTAB. Choline was chosen as the cation of choice due to the lower levels of toxicity measured when used (8). The effect that manipulating factors such as the anion chain length and ion ratio would have on the desired product, so a variety of anionic components were used for analysis. In all these syntheses, the anion used was a carboxylic acid that varied within its chain length. The product was a viscous liquid at room temperature with water and carbon dioxide as byproducts. A rotary evaporator and vacuum oven are used to decrease the water content, and a Karl Fischer titration is used to identify the final water content. Proton NMR spectroscopy is analyzed to confirm the final structure of the IL. The ILs used within this experimentation were synthesized and analyzed by fellow researchers.

Gold Nanorod Seed-Mediated Growth

Gold Nanoparticle Size and Charge Analysis.

A variety of carboxylic acids with varying chain lengths were chosen to serve as the anion for the IL synthesized. For each IL, the steps for synthesizing the seed solution included the addition of $HAuCl_4$ to the IL solutions and the addition of $NaBH_4$ under vigorous stirring conditions to reduce the gold. The steps for synthesizing the growth solution involved the manipulation of the volume of $AgNO_3$ to the IL before the addition of $HAuCl_4$, ascorbic acid, and

the seed solution. Each solution was then left for several hours before being removed and analyzed. It was initially unknown the effect that using IL in replacement of hexadecyltrimethylammonium bromide (CTAB) would have on the gold nanostructure. Therefore, the methods employed did not concentrate on creating structures of desired parameters, but they instead concentrated on analyzing the effects in comparison to other methods. The majority of this project involved the analysis of determining the initial possibility of the ILs as substitutes. Gold nanostructures are able to exist in a variety of geometries including as clusters, polycrystalline structures with quasi-spherical shape, single-crystal structures, nanoplates, and nanorods with anisotropic shapes (19). Each of these geometries display unique properties that separate their uses and compatibility. For biomedical uses such as PPTT, gold nanostructures with rod-like geometries are desired. Thus, it was important to determine if the synthesized products were of the correct geometry. The products were also analyzed by DLS to determine their size. The methodology used in this portion included three trial runs at a temperature of 25 °C. In these runs, the polydispersion index (PDI) was used to access the relative accuracy of the data as well as the shape of the distribution representing a Gaussian distribution curve Initial experimentation of this project did not discard candidates based on the size of the resultant product, but it instead allowed for analysis of IL effects and as a focus point for further work.

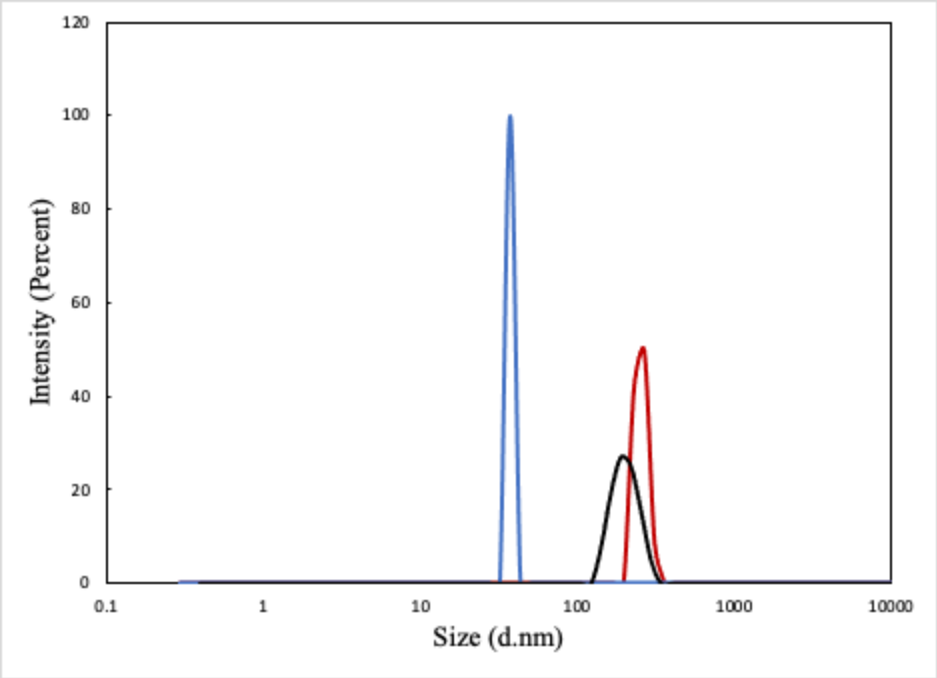

Figure 1: *Average nanostructure size changes dependent on alkyl chain length. Blue is 1:1 Choline:Pentanoate, black is 1:1 Choline:Octanoate, and red is 1:1 Choline:Hexanoate*

The size of the nanostructure is the most important factor in biomedical systems such as tumor targeting. Nanorods must be of certain dimensions to effectively and safely enter and clear biological systems through the renal system. The properties of gold nanorods in particular are also highly dependent on the size (19). Therefore, data on the size of produced nanostructures was measured by DLS analysis as previously denoted. As displayed by ***Figure 1***, the average size of nanostructure was highly dependent on the alkyl chain length of the anion. While it is seen that there is a general trend correlating longer alkyl chains to larger nanostructures, there are many exceptions that result in large differences from other alkyl chains that may differ in only one carbon. This indicates that each anion should be treated as unique and not be subject to being discarded due to results from other alkyl chains of similar length. The list of candidate ILs could

then be narrowed down by not further pursuing those that produced structures which were much too large. It should also be noted that none of these trials resulted in satisfactory UV-Vis data to confirm the synthesis of nanorod structure, and this is further explained below. However, this data led to the hypothesis that several other factors may be responsible for the determination of the structure's properties.

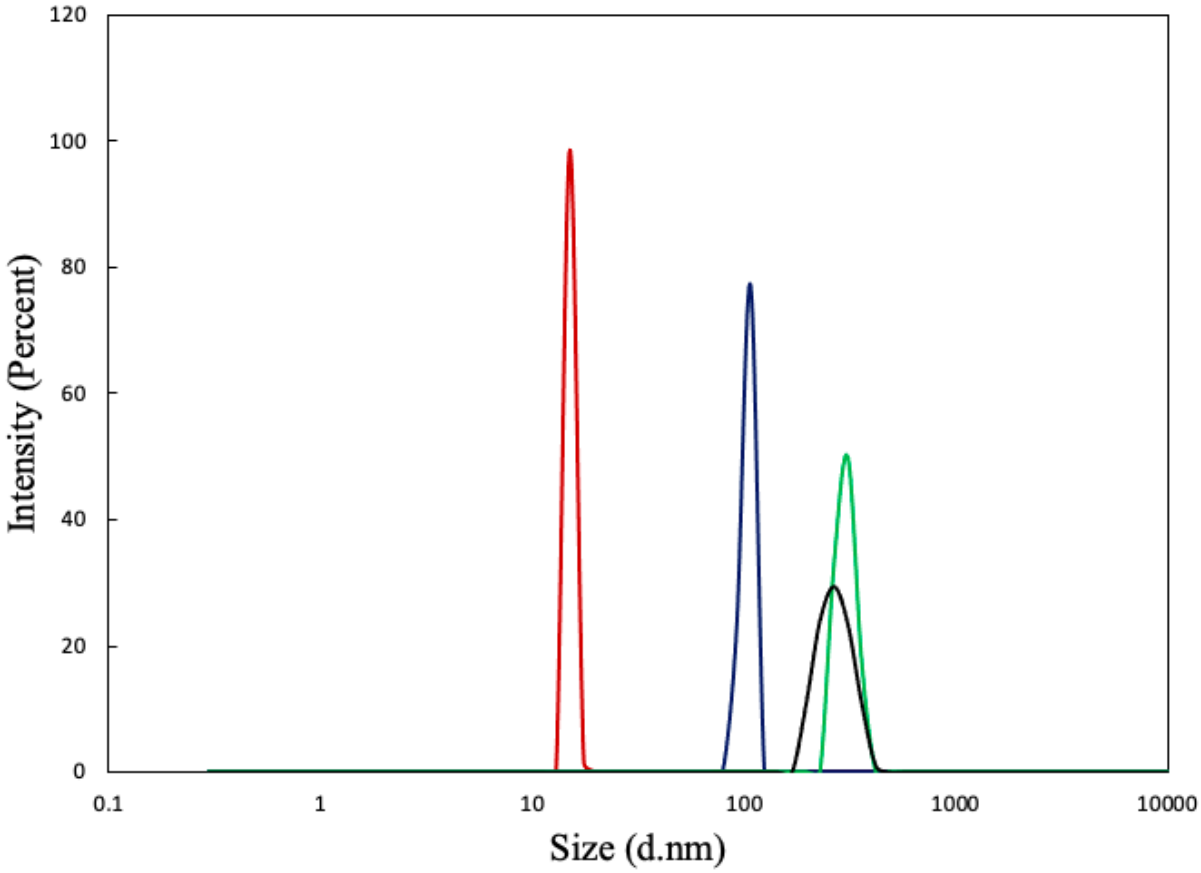

Figure 2: *The average size of the 1:1 Choline:Butyric Acid rod solution changes dependent on the volume of 40 mM silver used. Red is 10 mL, blue is 20 mL, green is 30 mL, black is 40 mL.*

It was previously discussed that the silver ions play an important role in directing the bilayer growth through underpotential deposition or Ag-Au co-deposition. Previous literature has

also indicated that factors such as the nanorod aspect ratios are dependent on the concentration of silver used. The aspect ratios are directly related to the LSPRs and they are highly size-dependent. For biomedical applications such as PPTT, the energy of these LSPRs being in the NIR is what allows for their practical application (23). This led to the hypothesis that manipulation of the silver concentration may lead to more favorable nanorod formation. As displayed by ***Figure 2***, the average size of the nanostructure produced was dependent on the concentration of silver. This further suggests that the size and geometry of the nanostructure produced is dependent on the silver concentration. The smaller size may be resultant from a limited amount of deposition and thus elongation. It is also possible that the formation of geometries such as nanospheres is favored without sufficient silver ions. In these scenarios, it is most likely that, if any, only a small amount of nanorods will be produced, and some form of centrifugation and filtration will be needed. The smaller differences between larger sizes and concentrations suggests that there is also a limit to the optimal silver concentration. This data shows the need for future work in optimizing the silver concentration used with candidate ILs. It also suggests experimentation that did not result in satisfactory formation of nanorods may be a result of an insufficient or excess silver concentration.

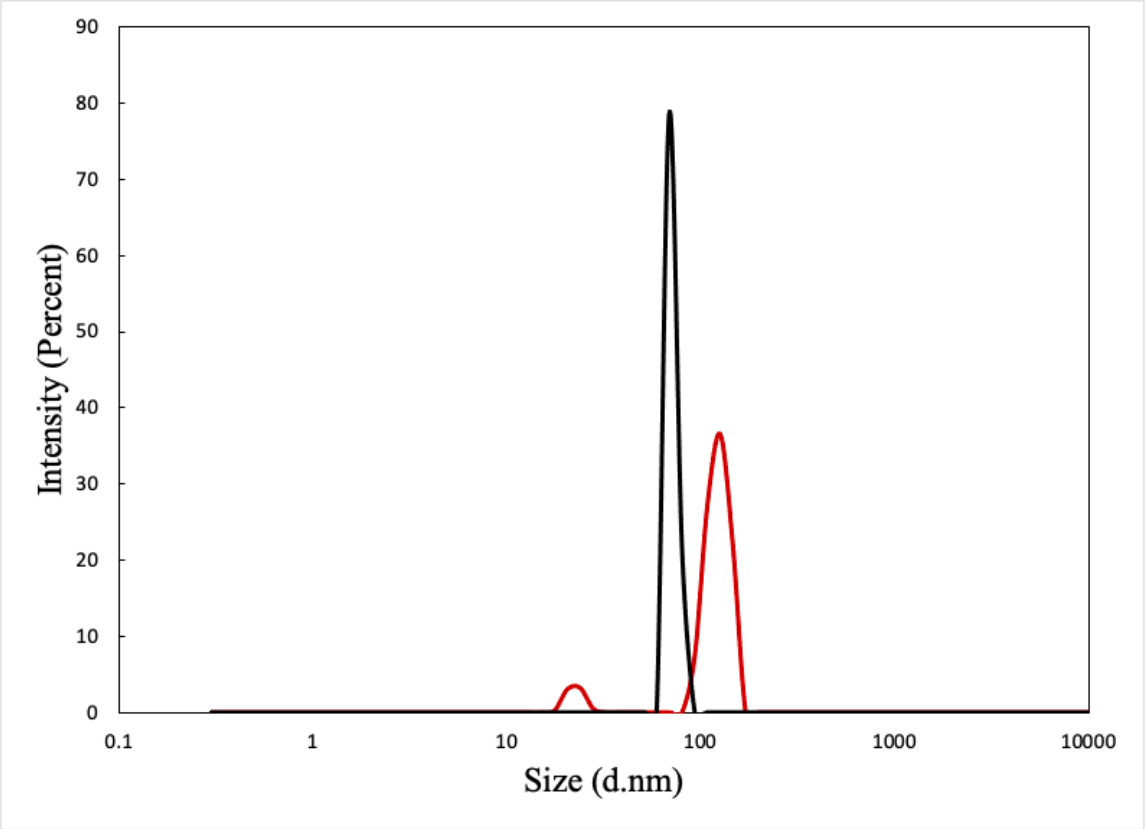

Figure 3: *The absence of $NaBH_4$ affects the nanostructure populations of 1:1 Choline:Pentanoate. Black is the rod solution created with the addition of the seed solution, red is the rod solution created without the addition of the seed solution.*

In the typical methodology, an agent such as a $NaBH_4$ is used to reduce the gold NP species strongly and quickly into seeds in the seed solution. The growth solution also contains ascorbic acid that is used to mildly reduce the gold species and promote directional growth. As previously mentioned, it was theorized the IL could serve as the surfactant in replacement of CTAB. The cation and anion within the ILs used also contain choline and carboxylic acids, so there is potential for competing pathways for reduction and deposition existing in solutions that contain

both components. Therefore, it was necessary to investigate the effects on nanorod formation without these components. Trials were attempted that used the same methodology as before except for the inclusion of the seed solution, and the results are displayed by ***Figure 3***. As displayed, the solution without the seed contained two populations of different sizes. However, the solution with the seed contained a single, more uniform peak. This data suggests multiple pathways that require more investigation. The data resulted from trials that included ascorbic acid as a mild reducing agent. The two populations thus could either be from reductions caused by the IL or by the ascorbic acid. It is also possible that this interplay depends on the relative strength of the acid used within the IL compared to the ascorbic acid, so differences in the trend may result dependent on the IL composition. Therefore, more trials are needed in order to accurately gather information about the mechanisms seen.

Zeta potentials were also analyzed for the different solutions created. This term dictates the degree to which charge aggregates on a structure's surface. Nanostructures with zeta potentials of charges greater in magnitude than ± 30 mV are generally predicted to be more stable. It can also give researchers an idea about particle aggregation (31). The research conducted in this experiment showed a large range of magnitudes that varied in signs. However, this is expected due to the complex interactions of the IL coated surface of the nanostructure produced. While this information is not used to necessarily rule out potential ILs as candidates, it is able to give more insight behind the potential mechanism for the resultant nanostructure formation.

Ultraviolet-Visible Spectroscopy Analysis

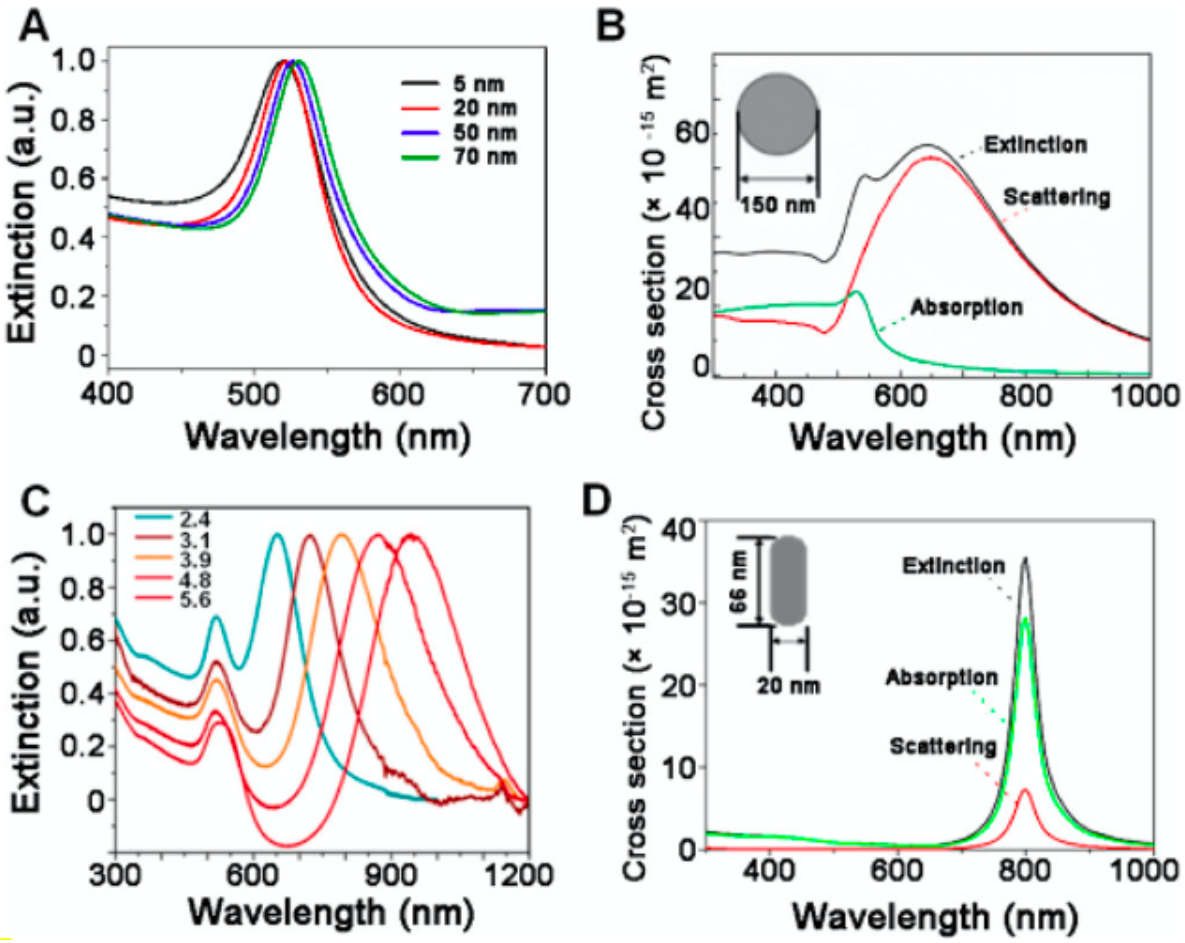

Figure 4: *Spectroscopic analysis shows visible differences between spectra received from gold nanospheres to that of gold nanorods. Spectra A and C are UV-Vis data while spectra B and D are the calculated LSPR spectra. Spectra A and B represent gold nanospheres while spectra C and D represent gold nanorods. This figure comes from research performed by Yang et al.* (19).

Colloidal gold can form a large range of geometries dependent on the chemical conditions to which it is subject. Each of these geometries displays chemical and thermophysical properties that are unique to their structure. For the purposes and future applications of this

research, the primary focus was to develop gold nanostructures that are cylindrical or rod-like in shape. However, the potential effects of using ILs in replacement of traditional surfactants and reducing agents on the geometry of the produced nanostructure was unknown. It was thus imperative to use spectroscopic methods to identify the potential shape of the produced nanostructure. The absorption spectrum of gold nanoparticles is well documented to show effects dependent on its size and shape (32). ***Figure 4*** compares the spectroscopic data of gold nanospheres and nanorods received from their UV-Vis spectra to their calculated LSPR spectra. This figure indicates clear, visible differences between the UV-Vis spectra dependent on the geometry of the NP. In general, gold nanospheres have one well defined peak while gold nanorods have two. While not a final confirmation, the UV-Vis spectra obtained in this research could thus be used to suggest or deny the desired rod-like geometry obtained.

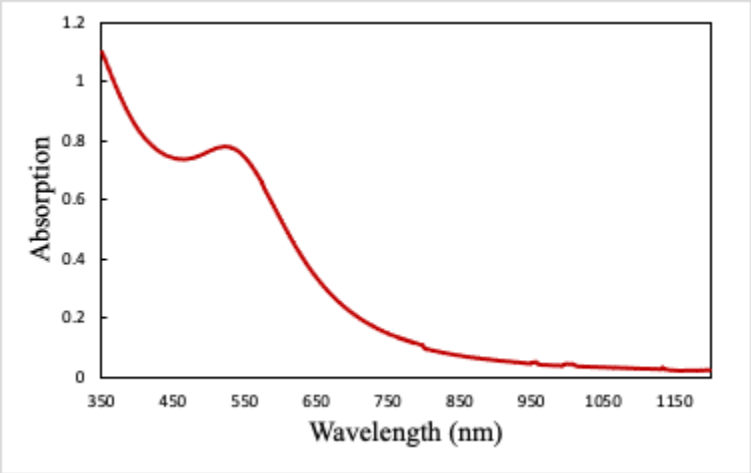

Figure 5: *The UV-Vis spectrum of the 1:1 Choline:Dodecanoate rod solution displays features characteristic of gold nanosphere formation*

Unfortunately, all the data obtained across the trials contained the same characteristics as those represented by ***Figure 5***. The spectrum contains one identifiable peak in the wavelength

range of 500 to 500 nm. This data indicates the synthesis proposed led to the formation of gold nanospheres instead of the intended nanorods. Despite the experimentation from this work being unsuccessful, the use of ionic liquids in the formation of gold nanorods is still achievable. As previously discussed, the mechanism behind nanorod formation is a complex interplay of many different factors. Each of these factors is further complicated when considering the multitude of possible ILs that can be used in replacement of CTAB. The formation of gold nanorods is a sensitive process that can be altered by even small amounts of impurities. In addition to this, each IL used may contain different chain lengths, ion ratios, or concentrations used within the experiment, and it has been shown the manipulation of any of these factors can lead to large differences that may ultimately determine the outcome. The concentration of both the silver ion and the ascorbic acid have also both shown to have large impacts that may need further experimentation and tuning for each IL candidate. It is also possible that the synthesis led to some formation of nanorods, but the yield was too low to be determined by this method. Further research may need to emphasize filtration techniques to effectively analyze different components on this scale.

CHAPTER 4: CONCLUSIONS

Modern medicine has evolved in countless ways to allow for the development of safer and more effective treatment options. The purpose of this research was to develop methodology that would result in the synthesis of gold nanorods using ionic liquids in replacement of CTAB, and this would allow researchers to further pursue one of these promising advancements. Unfortunately, this research requires further investigation to reach this goal, but it has highlighted many of the challenges faced and potential solutions in doing so.

While gold nanorods show potential for a variety of uses within biomedicine, they face toxicological concerns that are due to the use of CTAB. It is known that ILs can serve similar roles to that of CTAB while maintaining much lower levels of toxicity. Standard seed-mediated methods were then altered to develop a way to synthesize gold nanorods under these parameters. However, multiple methodologies were attempted and failed, but the results did provide insight for future experimentation that could prove successful. The synthesis proved to be resultant from many complex interactions, and the size of the nanostructures produced showed to be dependent on the IL's anionic component, silver concentration, and reducing agents used. It is possible that any of these factors could be the key to successful synthesis. The size of the nanostructure produced is important for future experimentation to determine their applicability to biological systems, but the main determination of success was based on if the resultant nanostructure was rod-like. This was determined by UV-Vis spectroscopy to identify characteristic peaks, and all of the results unfortunately indicated the structure to be spherical.

One of the main challenges faced in the experimentation is the lack of understanding behind the mechanism that produces rod-like nanostructures. The exact mechanism by which the surface is elongated is not understood, and it is thought to be a series of complex interacting

thermodynamic and kinetic pathways. There are multiple mechanisms proposed for each element in the synthesis, and the outcome of manipulating variables may be different depending on the mechanism adhered to. It is thus important to gain insight behind the mechanism to better understand controlling the synthesis, an aspect which is vital for implementation in biological systems. It has been shown that size determinations can be controlled, and further testing can be done to produce results that control the determinations of geometries.

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