University of Waterloo
Faculty of Mathematics

Optimizing Conditions of Ag-TA-CNC Solution Synthesis for Better Yield and Antimicrobial Properties

University of Waterloo
Faculty of Chemical Engineering
Waterloo, ON

Prepared by:
Anna Borbála Kis
2B Pure Mathematics
ID 20770526
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Memorandum

To: Emilie Montreuil Strub and Alyssa Oppertshauser, PD11 TAs
From: Anna Borbála Kis
Date:
Re: Work Term Report: Optimizing Conditions of Ag-TA-CNC Solution Synthesis for Better Yield and Antimicrobial Properties

Dear Emilie and Alyssa,

The following report contains an analysis of the procedure I used while working as a nanochemical research assistant at the University of Waterloo after my 1B term in Spring 2019, during which time I performed research on the functionalization of cellulose nanocrystals with silver nanoparticles for antimicrobial applications. A recommendation for an optimized procedure for the preparation of the solution will be contained within the report, based on numerous factors, namely yield and antimicrobial properties.

This report, titled “Optimizing Conditions of Ag-TA-CNC Solution Synthesis for Better Yield and Antimicrobial Properties” has been prepared for the PD11 course I am enrolled in following the completion of my 2B term in Pure Mathematics. This report is written entirely by myself, with information compiled from various resources and cited accordingly.

Thank you,

Anna Kis

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Executive Summary

The following report is concerned with a study centered around a green procedure for the synthesis of silver nanoparticles from bulk silver, and how to graft them onto cellulose nanocrystals using tannic acid. The study aims to create an antimicrobial solution which can be incorporated into latex for industrial applications. The purpose of this report is to determine the best procedure for the preparation of this solution by optimizing procedure conditions. This is done by manipulating solution pH and the molar ratio of tannic acid to silver ions.

The following report contains a brief introduction with a literature review followed by an analysis section containing a presentation and explanation of results. The section concludes with a discussion of said results in order to determine the optimal conditions. Finally, the conclusion summarizes the implication of the results, that a pH of 8 is optimal, and MR 0.05 is tentatively the best solution, and highlights further considerations and recommends next steps for testing.

1.0 Introduction

Professor Michael Tam's laboratory at the University of Waterloo within the Faculty of Engineering aims to conduct research on cellulose nanocrystals (CNC) and their potential applications in sectors such as healthcare, agriculture, waste management, and more. The following report will center around one of the research projects conducted within this laboratory. The aim of this project was to use silver nanoparticles, AgNP, grafted onto cellulose nanocrystals, CNC, to design surgical gloves with antibacterial properties and enhanced mechanical properties.

Since silver is an expensive resource and this project aims to have industry-scale applications, it is important that the procedure for the preparation of the antimicrobial solution, Ag-TA-CNC, where TA is tannic acid, be optimized for both yield and antimicrobial properties. The following section will briefly present a literature review, and an overview of the procedures used.

Following the introduction the analysis section will present characterization results from the various procedure conditions used. The aim of this report is to determine how experimental conditions present during the preparation of AgNP from bulk silver using TA impacts the resulting AgNP size and yield in order to determine optimal procedure conditions. The conditions which were manipulated was the pH of the solution, and the molar ratio of TA to Ag+.

1.1 Literature review

This study utilized tannic acid (TA), a plant polyphenol, chosen due to its similarity to polydopamine, for the green synthesis of silver nanoparticles (AgNP) from bulk silver ions (Ag+) at alkaline pH using methods similar to those found in a study by Sivaraman et al. (2009). Cellulose nanocrystals (CNC) are crystalline rods derived using hydrochloric or sulfuric acid hydrolysis from bulk cellulose (Tang et al., 2017). They were used in this study due to their high surface area for an efficient reaction surface; mechanical strength for the enhancement of the mechanical properties of latex; surface hydroxyl groups, and biodegradability (Tang et al., 2017). As well, CNC has been found to lead to a fourfold enhancement of the antimicrobial properties of AgNP (Shi et al., 2015).

TA reduces Ag+ to AgNP by hydrolysing to gallic acid (GA), which reduces Ag+, and glucose, which stabilizes it (Sivaraman et al., 2009), and is able to coat CNC to provide a site for AgNP attachment (Hu et al., 2017). The conditions under which the TA-AgNP reaction occurs may influence the size of the resulting AgNP: a study by Osawa and Walsh (1993) showed that until a pH 10, increasing pH increases GA yield, thus increasing the rate of Ag+ reduction and decreasing its size. As well, a smaller molar ratio (MR) of TA to Ag+ has been shown to decrease AgNP size and increase the rate of deposition onto the CNC surface, with an MR of 0.05 being ideal due to the ability of TA to donate 20 electrons (Taheri, 2018). Smaller AgNP particles are believed to exhibit better antimicrobial properties due to their increased surface area and easier bacterial cell wall penetration (Raza et al., 2016).

A review by Marambio-Jones and Hoek (2010) cites three potential mechanisms by which AgNP and Ag+, which are thought to behave by the same mechanisms, act as antimicrobial agents, although these are not completely understood:

1. AgNP is oxidized to Ag+ by oxygen in basic conditions, or hydrogen ions in prokaryotic cell membranes. The Ag+ enters the cells and disrupts ATP production and DNA replication.
2. AgNP damages the cell membrane by interacting with and penetrating the bacteria.
3. AgNP and Ag+ catalyses and generates reactive oxygen species. These form free radicals causing oxidative stress, resulting in DNA and membrane lipid damage.

1.2 Procedures

Taken from procedures by J. Jardin (personal communication, 2019).

1.2.1 Ag-TA-CNC Preparation

1.2.1.1 Varying pH

1. Add 5 g CNC to 500 mL of water. Homogenize the solution for 10 minutes followed by probe sonication for 5 minutes, and then bath sonication for 20 minutes.
2. Place beaker on a mechanical stirrer, and stir at 200 rpm.
3. Add 1.2 g 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) to the CNC dispersion.
4. Separate into five 100 mL batches and adjust the pH to 4, 6, 7, 8, and 10.

5. Add 0.50 g tannic acid to 20 mL of water and sonicate for 5 minutes.
6. Add 4 mL into each batch.
7. Allow TA-CNC solution to rest at room temperature overnight. (= 17h)
8. Measure and adjust pH.
9. Weigh 500 mg $AgNO_3$ and add it to 200 mL of DI water. Add the solution to the CNC dispersion.
10. Perform dialysis for three days
11. Store saved solutions in a beaker out of light, in a foil-wrapped bottle.

1.2.1.2 Varying MR

1. Add 4 g CNC to 400 mL of water. Homogenize the solution for 10 minutes followed by probe sonication for 5 minutes, and then bath sonication for 20 minutes.
2. Place beaker on a mechanical stirrer, and stir at 200 rpm.
3. Add 0.96 g 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) to the CNC dispersion.
4. Separate into four 100 mL batches and adjust the pH to 8.
5. Add 0.40 g tannic acid to 16 mL of water and sonicate for 5 minutes.
6. Add 4 mL to each batch.
7. Allow TA-CNC solution to rest at room temperature overnight. (= 17h)
8. Measure pH and adjust to 8 if needed.
9. Weigh 500 mg $AgNO_3$ and add it to 200 mL of DI water.
10. Mix solutions with TA:AgNO3 MR of 1.5,1, 0.5, and 0.05 by adding 2.560, 3.839, 7.681, and 76.810 mL of AgNO3 solution

11. Perform dialysis. Wash until waste stream is clear, then perform a last wash with MQ water.
12. Store saved solutions in a beaker out of light, in a foil-wrapped bottle.

2.0 Analysis

In order to draw conclusions about the efficacy of each parameter, the size, stability, and abundance of particles in the resulting Ag-TA-CNC solution was analyzed. The following section will present and briefly explain and analyze these results. To characterize the solutions, UV-vis spectroscopy and a dynamic light scattering (DLS) machine was utilized. Note that the presence of non-grafted substances was avoided by washing the solution.

2.1 UV-vis Spectroscopy

The success of depositing TA onto CNC, followed by AgNP onto the TA-CNC compound, was determined using UV-vis spectroscopy. This machine determines the amount of light energy absorbed by a substance, with increased (relative) absorbance implying a greater quantity of said substance (Mettler-Toledo GmbH., 2015). Different compounds should exhibit peaks at different wavelengths somewhere within the 200-800nm range; specifically, CNC is expected to peak at around 240nm, TA between 250nm and 300nm, and silver around 400nm (Sivaraman et al., 2009).

2.1.1 Varying pH

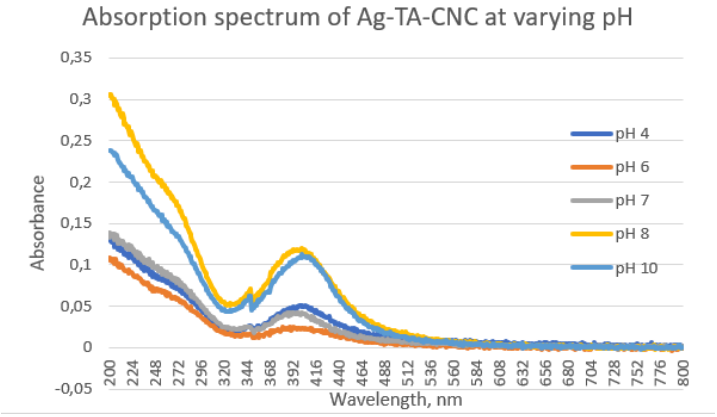

Figure 1: UV-vis spectroscopy results for Ag-TA-CNC at varying pH at 0.1 mg/mL (J. Jardin, personal communication, 2019)

The above graph indicates that the Ag-TA-CNC compound has been formed successfully. Furthermore, the greatest absorbance can be observed at pH 8, implying that it is the most successful pH for this procedure. This pH is the one often cited in literature for reducing Ag+ with a plant polyphenol, as well. Due to these, and the DLS machine, results, the MR optimization was carried out at pH 8.

2.1.2 Varying MR

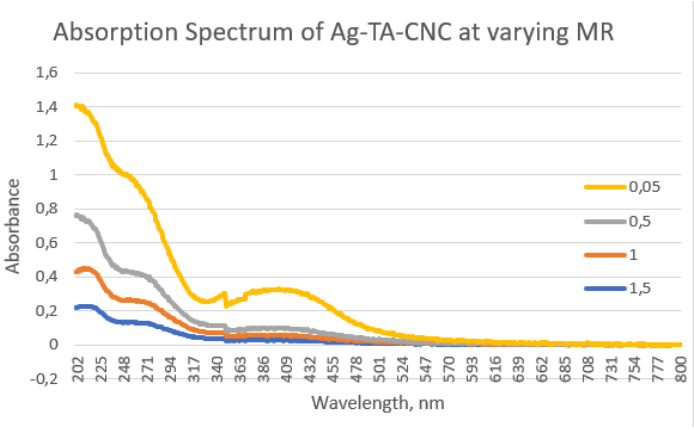

Figure 2: UV-vis spectroscopy results for Ag-TA-CNC at varying MR (J. Jardin, personal communication, 2019)

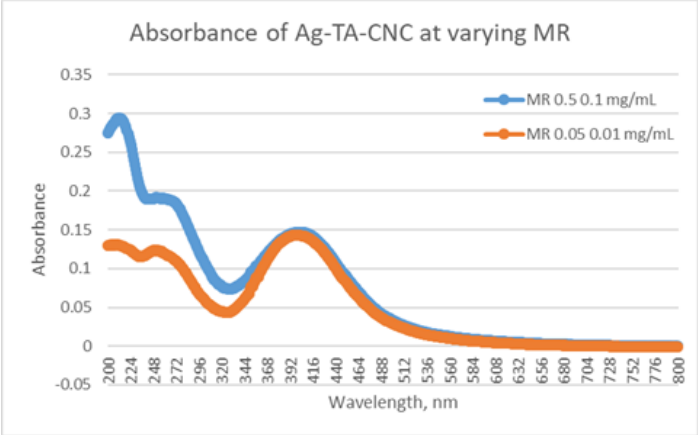

Figure 3: Diluted UV-vis spectroscopy of Ag-TA-CNC at MR 0.5 and 0.05 (J. Jardin, personal communication, 2019)

Figure 2 shows that the absorption for MR 0.05 is significantly higher than at the other MRs, implying that it is the optimal MR for this procedure. In order to get a more accurate reading, as the machine becomes inaccurate above an absorbance rating of 1, the two contending MRs were

diluted and re-graphed in figure 3. The only way to accurately fit both onto the same graph was to dilute to different degrees. Clearly, figure 3 further implies that MR 0.05 is the superior ratio.

2.2 DLS

The DLS was used to determine the zeta size and potential of the compounds.

2.2.1 Zeta potential

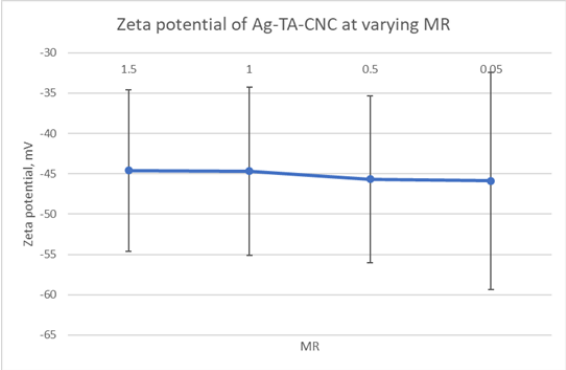

Figure 4: Zeta potential (mV) of Ag-TA-CNC at varying MR (J. Jardin, personal communication, 2019)

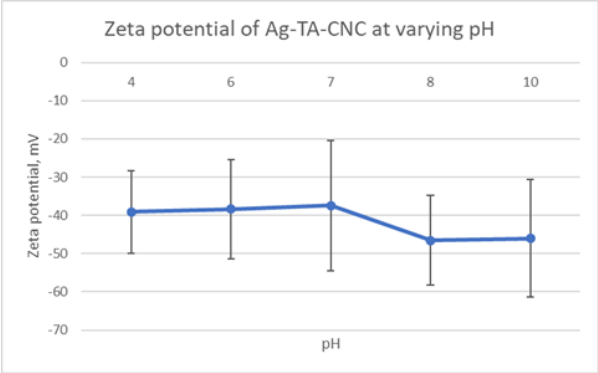

Figure 5: Zeta potential (mV) of Ag-TA-CNC at varying pH (J. Jardin (personal communication, 2019)

Figure 4 and 5 indicate that the compound is stable at all MRs and most pHs, save for pH 7, since a zeta potential of less than -30 mV indicates stability, although the large, overlapping error bars make drawing conclusions difficult.

Another batch of the solution was prepared at pH 8 with MR 0.5 and 0.05. Their respective zeta potentials were -48.9 ± 13.3 and -46.8 ± 10.5. While not very precise, all these values are needed for is to confirm the stability of the compound.

2.2.2 Zeta size

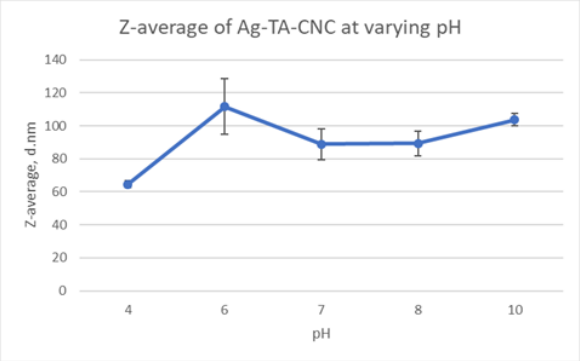

Figure 6: Z-average (d.nm) of Ag-TA-CNC at varying pH (J. Jardin (personal communication, 2019)

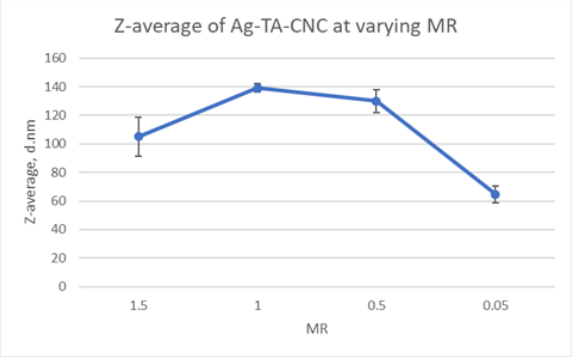

Figure 7: Z-average (d.nm) of Ag-TA-CNC at varying MR (J. Jardin (personal communication, 2019)

Surprisingly, figure 6 implies that pH 4 yields the smallest particles, and the size at pH 6 seems to be an outlier in the observed trend of increasing size with pH. Figure 7, on the other hand, seems to confirm that MR 0.05 yields the smallest particle size. Finally, the later batch of MR 0.5 and MR 0.05 at pH 8 had a Z-average, in d.nm, of 82.26 ± 4.10 and 25.34 ± 5.58, respectively.

2.3 Synthesis

The following section will analyze the findings mentioned above.

First, the UV-vis spectroscopy indicated that the procedure was successful in synthesizing the Ag-TA-CNC compound. As well, as implied by the literature, absorbance was greatest at a pH of 8, indicating that this is the optimal pH for this procedure, potentially due to the rate of TA hydrolysis. However, the absorbance spectrum is only able to indicate grafting success. The zeta potential of the particles was used as a confirmation of their stability. Due to the degree of the error bars, no real conclusions could be drawn, as the overlap in results did not allow for a general trend to be observed. More analysis in the future would be necessary. As well, to further document the compound's stability, studying how absorption decreases with time would be necessary. Such findings are important for industrial applications: the produced compound should not only be effective and efficient, but it should have a long shelf-life for it to be scalable to industry. As well, the Z-size results imply particle size increases with pH. However, in order to be able to draw any proper conclusions TEM must be conducted, especially because it is difficult to determine how the amount of TA grafted onto the CNC surface influences the particle's size; it may not actually be the AgNP size that is changing.

Similarly, the UV-vis spectroscopy indicated that the ideal MR is 0.05 between TA and Ag+. Unfortunately, this is a 1:20 ratio, meaning that significant amounts of Ag+ are necessary. On an industrial-scale, this may be quite expensive. As such, further testing will be necessary in order to determine if MR 0.05 yields significantly better results than the other MRs; if not, such an increase in cost may not be worth it. Such conclusions may be drawn by mechanical testing after incorporation into latex, antimicrobial properties, stability over time, and more.

However, it is important to keep in mind that while the amount of substance grafted increased, the particle size decreased, as indicated by the Z-average (of course, this must be confirmed with TEM). While this is hypothesized to yield better antimicrobial results, it does mean that in order to utilize the same weight percentage of compound, a lot more of the solution must be prepared when compared to the other MRs. This is further supported by dry-weight results indicating significantly lower dry weight at MR 0.05. As such, the prepared latex may become "watered-down," or simply too great quantities of the solution must be prepared. This may become cumbersome, expensive, or infeasible. Such an issue may occur because of the small particle size: it is possible that a lot of the product is lost during the washing process by diffusion through the membrane. Alternatively, more osmosis may occur resulting in a more dilute solution because of the increased particle amounts. However, more analysis, and a better method of washing, is needed in order to draw any real conclusions.

Finally, the zeta potential of the different MRs indicates general stability. As mentioned before, this is merely a confirmation; further testing through repeated UV-vis spectroscopy over time will be necessary.

3.0 Conclusions

The purpose of this report was to determine the optimal conditions for the synthesis of Ag-TA-CNC by varying the pH and MR between TA and Ag+. While more experimentation will be necessary to completely understand the effect of MR and, more specifically, particle size on the substance's antimicrobial properties, and how its combination with latex affects the rubber's mechanical properties, the characterization results from the study indicate that a pH of 8 is optimal. This conclusion is further backed by the literature. As such, this pH was chosen when moving forward with the MR and subsequent studies. While the MR study seems to indicate that a MR of 0.05 is ideal as it results in a greater absorbance and decreased particle size, it is unclear how such things, as well as the drastic decrease in the weight percent of the substance, may affect the solution's properties and applications. As such, further experimentation will be needed before a proper conclusion can be drawn. Furthermore, it is important to keep in mind the needs of the industry: while it is important that the proposed procedure produce the best results in terms of yield and antimicrobial and mechanical properties, the shelf-life of the produced particles, and the cost of production, must also be taken into account.

Moving forward, it is recommended to conduct TEM testing to better determine particle size. As well, antimicrobial tests, both using only the solution and the solution after incorporation into latex, should be conducted. Similarly, the mechanical properties of the latex, and the latex combined with various versions of the solution should be tested. Finally, the substance cost and benefits must be analyzed in order to determine the best solution for the proposed product.

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