2020

Synthesis of Polyacrylic Acid - Dopamine Nanoparticles as Radical Scavengers for Antioxidant Applications

Russell D. Cox
University of Central Florida

SYNTHESIS OF POLYACRYLIC ACID - DOPAMINE NANOPARTICLES AS RADICAL SCAVENGERS FOR ANTIOXIDANT APPLICATIONS

by

RUSSELL DAVID COX

A thesis submitted in fulfillment of the requirements
for the Honors in the Major Program in Chemistry
and in the Burnett Honors
College at the University of
Central Florida
Orlando, Florida

Spring Term 2020

Major Professors: Lei Zhai and Qun Huo

ABSTRACT

The antioxidant activity of novel drugs has been of increasing interest in recent years. Free radicals are linked as a cause to many diseases such as atherosclerosis and cancer,[1] so development of drugs that can scavenge and break down free radicals is needed. One such potential solution is using dopamine, which is water-soluble and an antioxidant. However, the tendency of antioxidant drugs reacting undesirably with proteins and other biochemical compounds is a big issue for the drugs' antioxidant potential. One solution is by encapsulating the antioxidant compound in biocompatible polymer nanoparticles. In this project, dopamine is bound to the polymer polyacrylic acid (PAA) and spherical PAA-dopamine nanoparticles were synthesized. Following their synthesis, the nanoparticles were characterized by Dynamic Light Scattering (DLS), Transmission Electron Microscope (TEM), and Fourier-Transform Infrared (FT-IR) spectroscopy and were shown to have an average size of 90 nm after dialysis cleaning. Finally, their hydroxyl radical (OH·) scavenging ability was tested through pH changes and fluorescence and the data acquired suggests possible radical scavenging potential.

TABLE OF CONTENTS

CHAPTER ONE: INTRODUCTION

Polymeric Nanoparticles

Polymeric nanoparticles (NP) have become a very promising avenue of research for scientists in the modern day and are incorporated into applications from energy and technology[1] to medicine[2] and in the food[3] and oil industry[4]. Nanoparticles are formally categorized as particles within the size of 10-1000 nm.[5] Typically, polymeric nanoparticles are prepared based on their desired use and the nanoparticle scaffold matrix can be created from a wide range of materials. The type of polymer material has an effect in the size, shape, solubility, stability, charge, permeability, and biological and environmental properties of the nanoparticles. Combinations of properties can result in complications in the particle synthesis and other undesirable traits such as particle aggregation. Meanwhile, other property combinations can result in adequate drug delivery systems by having optimal biocompatibility, biodegradability and chemical degradation.

In terms of synthesis, nanoparticles vary greatly in their simplicity of preparation. While some nanoparticles can be synthesized with very fast and easy methods, others require much more sophisticated and detailed synthetic schemes. Several typical methods for the formation of polymeric nanoparticles are by 1) either the solvent evaporation method or the spontaneous emulsification method, both caused by the dispersion of the performed polymers; 2) the polymerization of individual monomers; 3) the ionic gelation of hydrophilic polymers; or 4) the precipitation of the nanoparticles.[5]

Free Radicals and Antioxidants

The American diet has shown increasing amounts of fried foods and alcohol, both foods of which have been related to high amounts of free radicals.[6] Free radicals are reactive compounds with an unpaired electron that can donate or accept an electron. The free reactive electron encourages oxidation or reduction reactions and makes the free radicals highly sensitive to other molecules. In the organisms, the free radical compounds are capable of damaging cells and eventually leading to biological malfunctions. To address the problem, many scientists have turned towards foods high in antioxidants, examples of which include vegetables, fruit, and teas.[7,8] Good antioxidants are compounds that reduce the damage of free radicals by inhibiting oxidation[9] and are commonly found in nature. It is of interest, to talk about a common neurotransmitter found in the body called dopamine, this compound exhibits good antioxidant activity due to the dihydroxyl groups within the aromatic benzene ring (see figure 1).

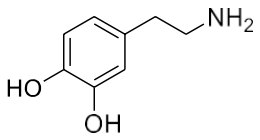

Figure 1. Dopamine, a hormone and a neurotransmitter responsible for many important roles in the body, which has also been successfully used in many nanoparticle applications due to its interesting chemical properties.

Furthermore, other similar compounds to dopamine have shown medicinal properties, an example of which is 3-hydroxytyrosine (3-HT); a common synthetic building block for many organic compounds with medicinal properties such as vernakalant[11], a specialized heart

medicine. These class of compounds (mainly known as catechol's) have also proven themselves to possess powerful anticancer, antimicrobial, and neuroprotective properties.[12-15] The dihydroxyl groups in these catechol compounds serve by donating H· radicals to the free radical compounds and therefore lower their high reactivity. The loss of the hydroxyl proton causes the electron to form a double bond between the oxygen and carbon atoms, which then produces a radical rearrangement that ends up in a stable quinone form (due to the intramolecular hydrogen bond within the phenoxy radical).[16] In our study, the antioxidant used is dopamine, a catecholamine which facilitates antioxidant activity due to it being able to stabilize itself into the quinone form. The proposed mechanism for the radical reaction and source of antioxidant activity is shown in **Figure 2**.

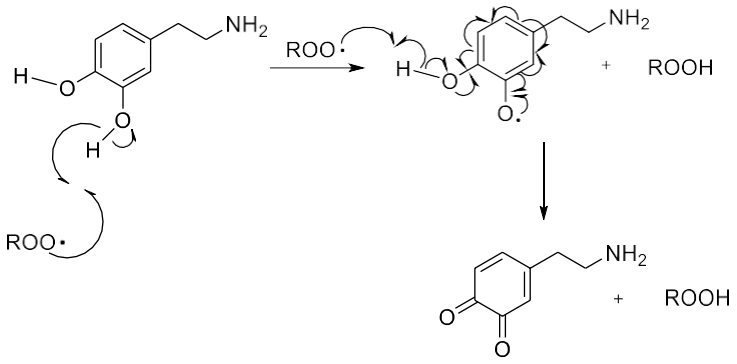

Figure 2. Proposed mechanism for the free radical reaction

Focus of Project

The objective of the project is to explore the viability of creating a nanoparticle of the polymer PAA and the antioxidant compound dopamine via the 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling reaction. The nanoparticle system is hypothesized to have similar or stronger antioxidant properties than free dopamine because there is a higher number of dihydroxyl groups within the formed nanoparticle. In addition, the nanoparticle's desired shape is that of a sphere with an interior antioxidant region and an exterior polymeric shell. A proposed polymer particle model can be seen in **Figure 3**.

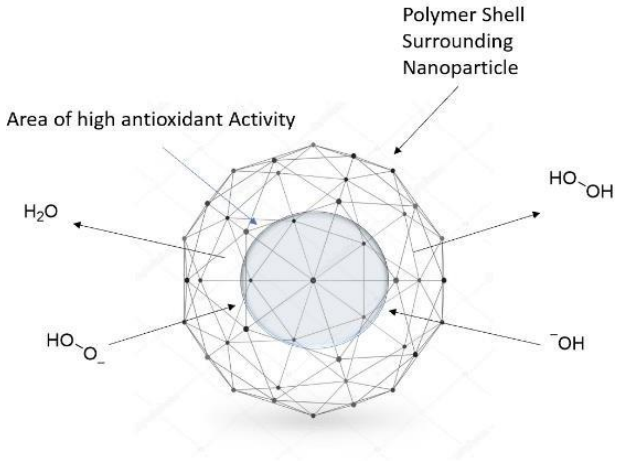

Figure 3. Desired nanoparticle spherical shape with an interior antioxidant core and an exterior polymer shell.

The model as described is desirable because the antioxidant rich core of the nanoparticle is essentially inaccessible to bigger compounds or proteins while still being accessible to smaller free radical species. The free radicals will be able to easily access the core and have their reactivity neutralized by the donation of an electron and proton, and the products leave the nanoparticle. Additionally, the PAA polymer chain is able to provide the nanoparticle with the desired stability.[45]

Furthermore, the driving force of formation for the nanoparticle is the London intramolecular forces which form the nanoparticle into a sphere. The aforementioned antioxidant properties in the proposed PAA-dopamine particle system could be utilized as a free radical scavenger in any synthetic, medical or industrial use.

CHAPTER TWO: METHODS

Nanoparticle Synthesis

In a 20 mL vial, 8 mL of deionized water and 0.1152g of a 25w/v% PAA solution (0.4mmol) were dissolved. 9.585mg of EDC (0.05mmol) from a 0.0521 M solution was added dropwise as the reaction was stirred. 9.482mg of dopamine (0.05mmol) was then added. The solution was stirred for 12h and left to settle for 4h. The reaction equivalence was 8:1:1 PAA to dopamine to EDC molar ratio. The product was then characterized by DLS to verify the synthesis. The solution was then loaded into dialysis tubing, and the starting material and any byproducts were removed after 3 phases of dialysis purification. The solution was lyophilized for 48 hours to obtain solid powder product. Finally, the solid product was resuspended in a 10mg/mL ratio and characterized by DLS, FT-IR, and TEM to obtain the information of nanoparticle composition and size.

Radical Scavenging Test through pH

Radical scavenging tests were performed by adding 1 mg-increments of the nanoparticles into a known concentration of H_2O_2 (50mM) in a solution of distilled water (pH 7) and measuring the resulting pH changes. Once sufficient data was obtained, a NP amount vs $[OH^-]$ plot was created. As a control, the experiment was repeated with pure PAA.

Radical Scavenging Test through Fluorescence

In multiple vials each containing 19.4mL of 25mM solution of DPBS, 30% w/v H_2O_2 was added and stirred thoroughly to create a 10mM H_2O_2 / 25mM DPBS solution. 1 mg of NP was added to each vial and the reactions were stirred for ~1hr, 1.66mg of terephthalic acid was

then added to each vial to create a 5 mM solution and stirred for 24h. The reactions were measured for fluorescence activity.

CHAPTER THREE: RESULTS

DLS Data

Table 1. Obtained dynamic light scattering data before and after dialysis.

	Trial 1 (before dialysis)	*Trial 2 (after dialysis)*
Count Rate (kcps)	108.5	81.5
Attenuator	11	11
PDI	0.501	0.486
Peak 1	106.5 (d. nm) 82.2%	90.97 (d. nm) 76.9%
Peak 2	14.79 (d. nm) 15.2%	16.02 (d. nm) 22%
Peak 3	4789 (d. nm) 2.7%	5208 (d. nm) 1.10%

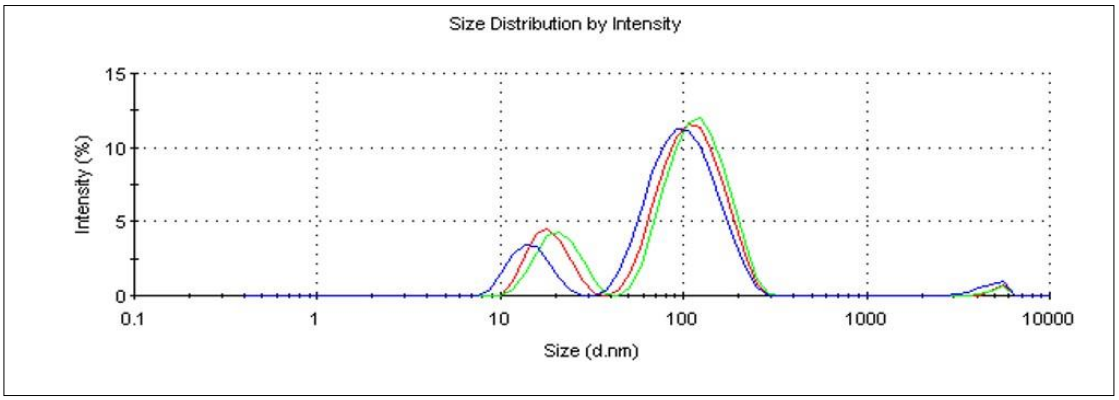

Figure 4. Dynamic light scattering size distribution by % intensity for the PAA-Dopamine nanoparticles before cleaning with dialysis.

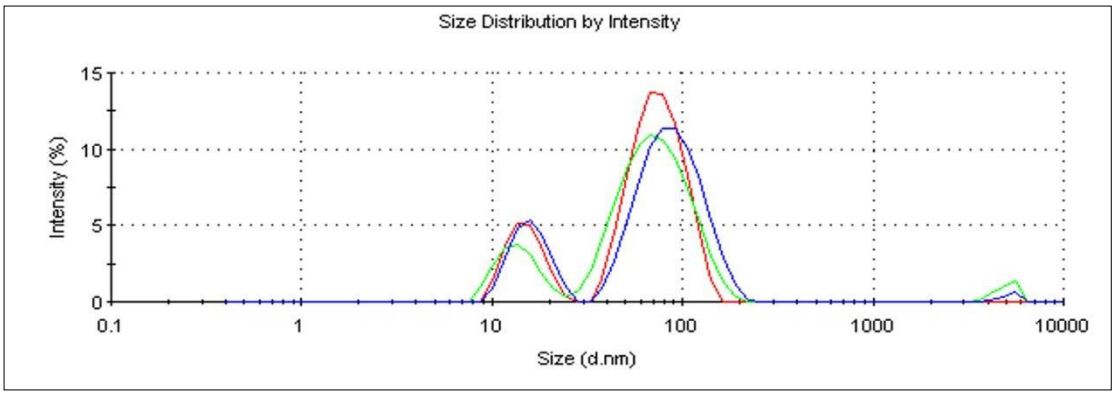

Figure 5. Dynamic light scattering size distribution by % intensity for the PAA-Dopamine nanoparticles after cleaning with dialysis.

FT-IR Data

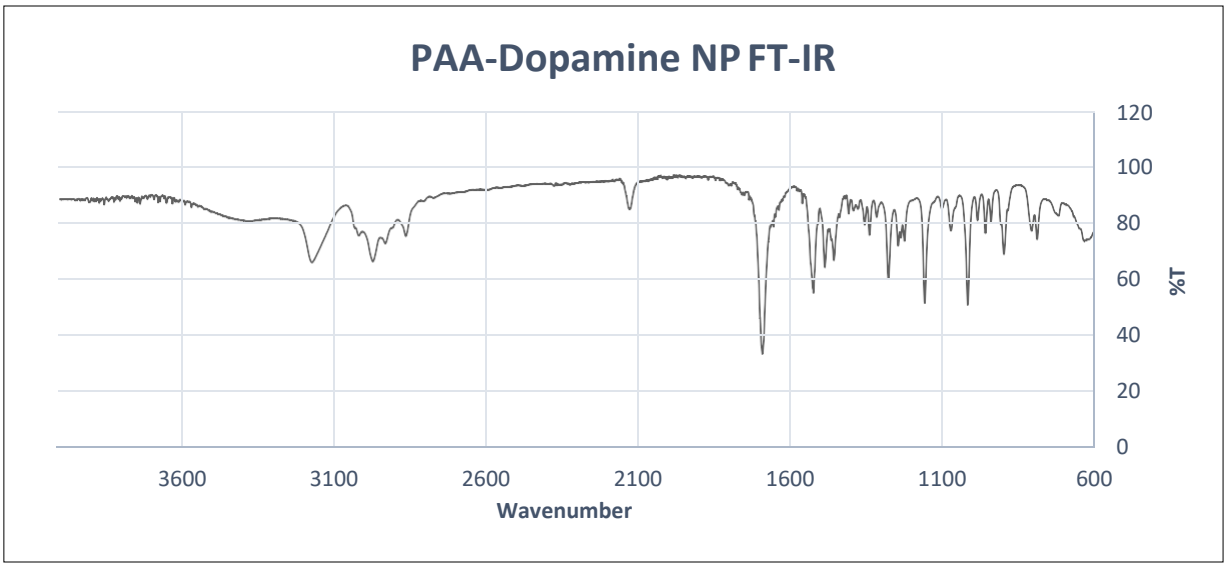

Figure 6. FT-IR spectra of the PAA-Dopamine NP after dialysis.

Fluorescence Data

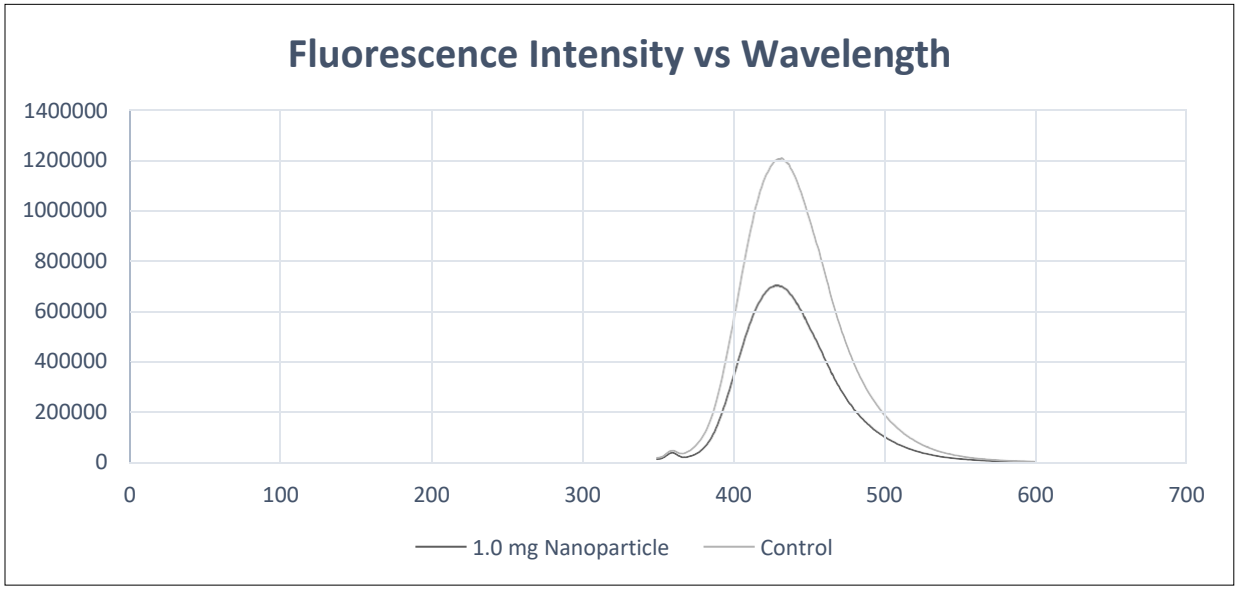

Figure 7. Radical Scavenging of OH· radical with 1.0 mg of the PAA/Dopamine NP vs a control (no NP). Note that the intensity in Fluorescence indicates more OH· radicals present.

pH Radical Scavenging Data

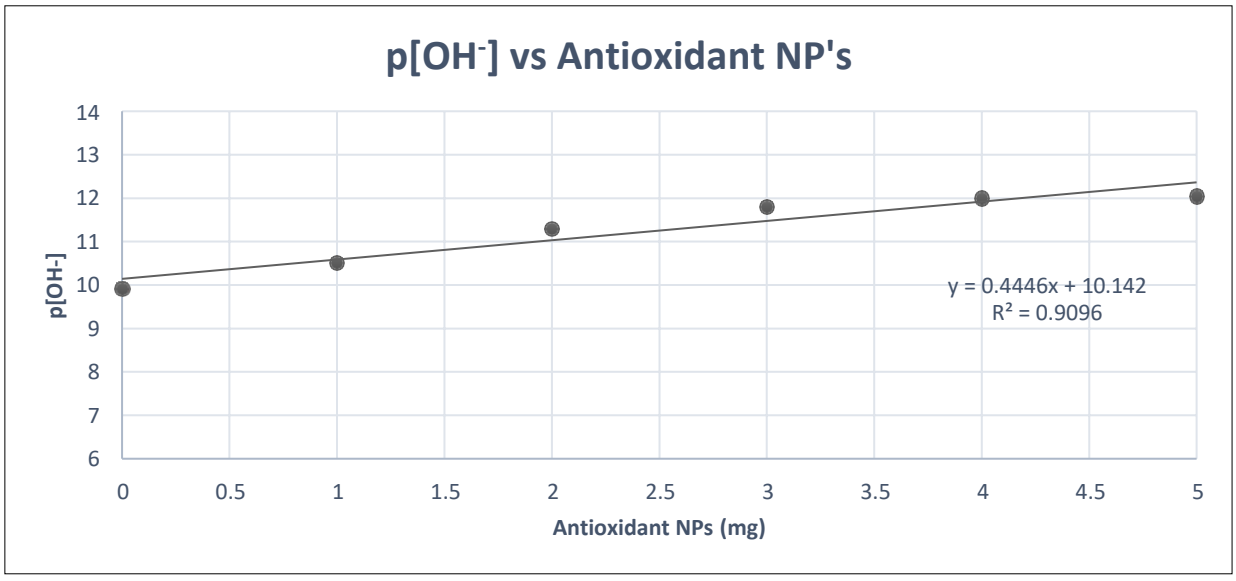

Figure 8. Plot of p[OH-] vs NP amount showing how the [OH-] decreases with increasing the amount of antioxidant NP.

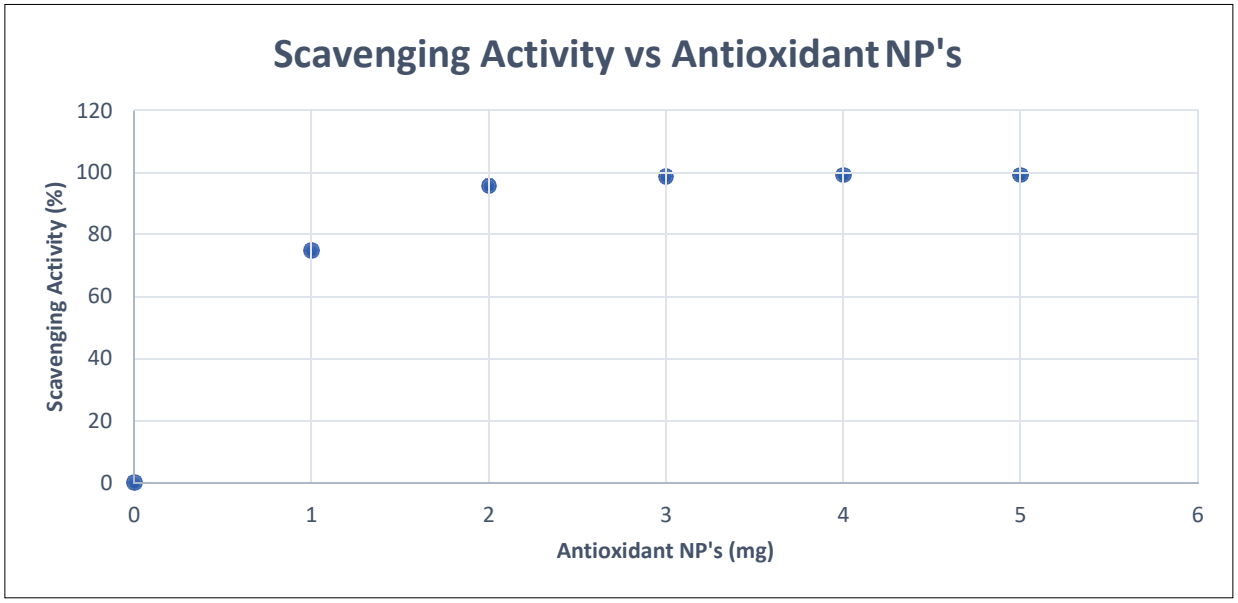

Figure 9. The plot of the OH^- scavenging activity (%) vs mg of NP indicates that as more mgs of the NP are added more OH^- molecules are scavenged.

Transmission Electron Microscopy Data

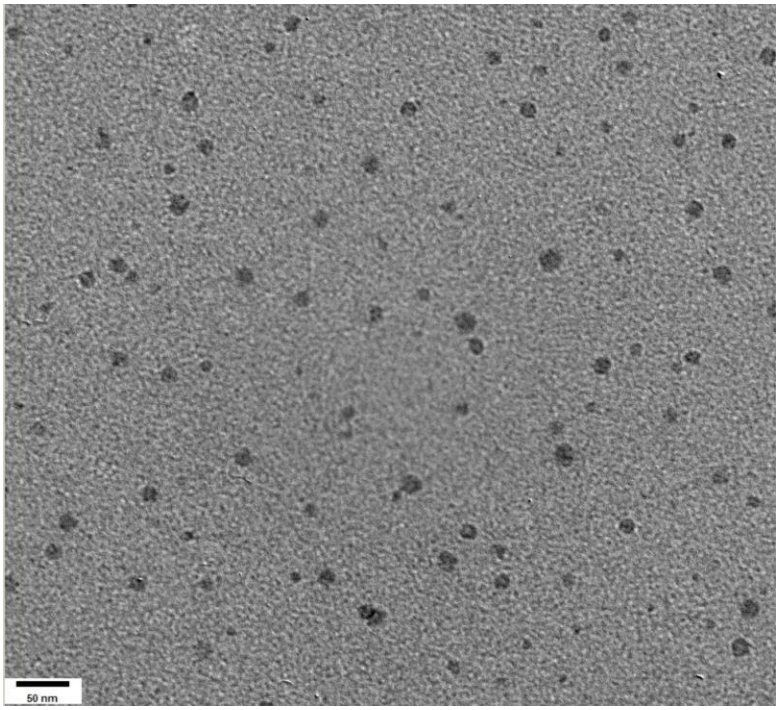

Figure 10. Transmission electron microscopy image of a PAA-Dopamine nanoparticles at 317.2kx magnification.

CHAPTER FOUR: DISCUSSION

Nanoparticle Formation

PAA has been reported in literature to have strong hydrogen bonding interactions.[17] Because the Van-der-Waals interactions of PAA in water have been estimated to be only about 7%,[18] the biggest factor determining the behavior of PAA in water is the PAA concentration. That is, the greater the distance between PAA and other molecules, the more it will behave like a polymer chain. However, if the polymer – substrate, or polymer - polymer intermolecular distance is short, the polymer will undergo curling due to the intermolecular forces of the two.[17] Furthermore, if the polymer concentration is too high, the result will be a hydrogel formed by the network of polymer chains. It is for this reason, that one of the biggest problems in the synthesis of polymer nanoparticles is the concentration of the respective polymers as a concentration lower than ideal would yield no nanoparticle formation and a concentration higher than ideal yields a hydrogel. In this project experimental evidence was found to confirm this claim as initial attempts saw the formation of hydrogels whereas later trials with 50 mM PAA concentration yielded nanoparticle formation. Additional parameters for the nanoparticle formation include incorporating different temperatures, stirring/mixing techniques, and reaction time. Studying these parameters may prove beneficial and help optimize the efficiency of the nanoparticle formation.

Characterization

Analysis of the synthesized nanoparticles with DLS showed an average size of 106.5 nm with an average count rate of 108.5 kcps before dialysis. After 3 phases of dialysis, the cleaned solution reported a size distribution of 90.97 nm and 81.5 kcps. The decrease in size and count

rate may be an indicator of a cleaner solution of suspended nanoparticles, however it is also possible that some nanoparticles were dissociated through the pressure formed by the diffusion gradient. Analysis of the TEM image shows a nanoparticle of roughly 15 nm in size. Even though DLS, is often used in addition with a microscopy technique (such as TEM or SEM) as the main method of verification for nanoparticle formation, comparisons between the two are often meaningless. This is because the DLS technique is able to give us size distribution ranges by measuring the doppler broadening as it is affected by the translational diffusion (also known as brownian motion) of the particles. On the contrary, the TEM technique works very differently by utilizing beams of electrons to image the surface of an object. Typically, particle size determination techniques give different results for particle sizes and their distributions due to the multiple dimensionalities.[19] It is for this reason that comparisons between different techniques are largely considered qualitative.

Finally, the FT-IR data of formed nanoparticle showed a carbonyl peak at 1687 cm^{-1}, and a peak at 1522 cm^{-1} typical of an amide.[19] The peaks at 2850-2950 cm^{-1} represent the alkyl chain of the polymer, while as the peaks at 3180 cm^{-1} and 2118 cm^{-1} are part of the C-H stretching, and C-H bending overtone of the aromatic ring. The two alcohol groups can be seen at ~3400 cm^{-1}

and their weak and broad appearance signals the fact that they are intramolecular bonded.[20]

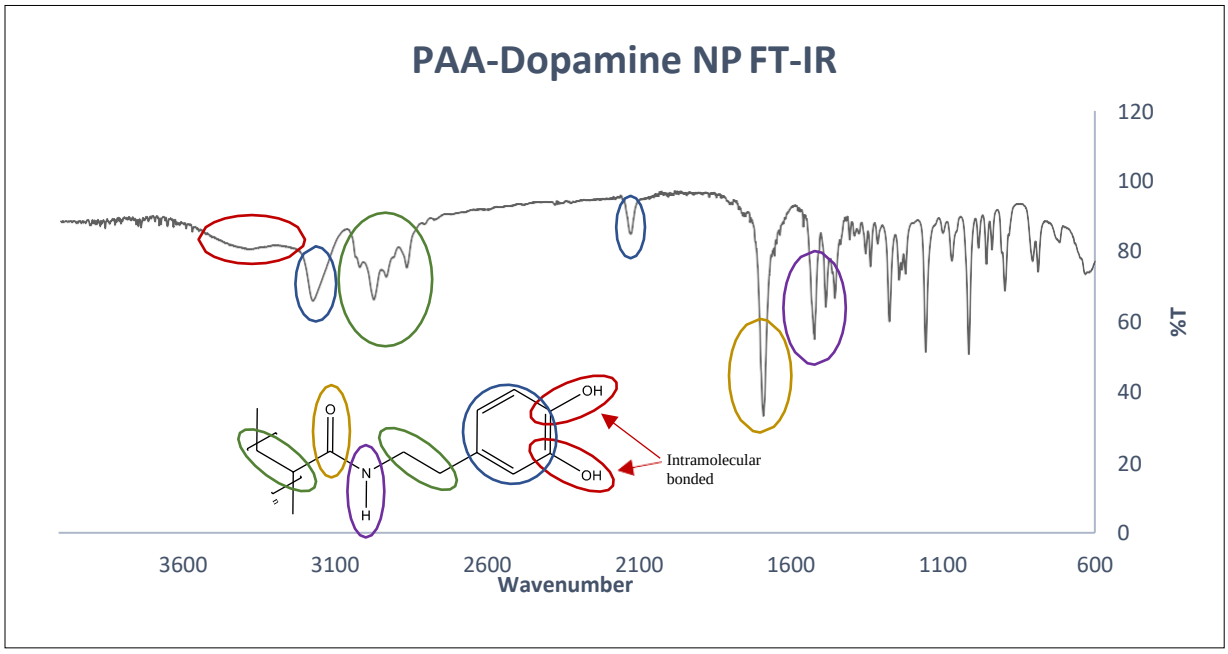

Figure 11. Analyzed FT-IR spectra of the PAA-Dopamine NP.

Scavenging Activity

In order to test the OH^- scavenging ability of the nanoparticle, the pH changes in a solution containing H_2O_2 were observed after addition, and prior to the addition of the antioxidant nanoparticle. Because H_2O_2 spontaneously dissociates into OH^- and many other free radical species, it provides a good way for testing the OH^- scavenging ability of the nanoparticle. Upon addition of small milligram increments of the PAA-Dopamine nanoparticle, the pOH^- of the solution can be seen to adjust following a linear relationship $y = 0.4446x + 10.142$ where x is the amount (in milligrams) of antioxidant nanoparticle added and y is the pOH^-. As observed by this relationship, the larger the number of nanoparticles in the solution, the higher the pOH^- which would then result in a smaller number of OH^- molecules.

In order to test the $OH\cdot$ radical scavenging abilities of the antioxidant nanoparticle, (as

compared to the OH^- scavenging properties) a fluorescence test was performed. Terephthalic acid is a common compound with very interesting properties. That is, the compound itself is not fluorescent, however, in the presence of OH· radicals, the compound becomes 2,5-dihydroxyterephthalic acid, a strongly fluorescent compound. After addition of the antioxidant nanoparticles in a solution rich in OH· radicals, some of the OH· radicals become neutralized by the nanoparticle. This results in a lower number of OH· radicals present which then translates to a lower fluorescence intensity, as seen in Figure 8. It is interesting to note that a 1.0 mg of nanoparticle added resulted in a decrease in fluorescence of 42%. This correlates to a 5.5430 mg of OH· radicals scavenged per milligram of nanoparticle; this test shows initial evidence for the ability of the synthesized nanoparticle to act as a OH· radical scavenger, however further tests to fully confirm these properties should be performed.

CHAPTER FIVE: CONCLUSION

In conclusion, PAA-dopamine nanoparticles were synthesized by a simple and robust method and demonstrated promising synthetic antioxidant activity. The nanoparticle system comprised of dopamine bound to the carboxylic groups in PAA via EDC coupling. The nanoparticle desired exhibits a spherical shape and its formation is driven by the hydrophilic and hydrophobic interactions with the solvent which due to the London dispersion forces would help drive its formation. In order to protect the antioxidant region of dopamine and increase its stability, the protective polymer shell is created to limit any interactions with compounds bigger than free radicals. The synthesized nanoparticles exhibited OH^- and $OH\cdot$ radical activity which makes it a viable option for further research as a possible antioxidant drug. Thus, a polymeric nanoparticle with good prospects to antioxidant activity was developed and could be utilized in medicine, total synthesis or many other industries.

REFERENCES

1. Bankson, D. D., Kestin, M., & Rifai, N. Role of free radicals in cancer and atherosclerosis. *Clinics in laboratory medicine,* **1993,** *13*(2), 463-480.

2. Raimondi, F.; Scherer, G. G.; Koetz, R.; Wokaun, A. Nanoparticles in Energy Technology: Examples from Electrochemistry and Catalysis. *ChemInform* **2005**, *36* (28).

3. Zhang, L.; Gu, F.; Chan, J.; Wang, A.; Langer, R.; Farokhzad, O. Nanoparticles in Medicine: Therapeutic Applications and Developments. *Clinical Pharmacology & Therapeutics* **2007**, *83* (5), 761–769.

4. Peters, R.; Dam, G. T.; Bouwmeester, H.; Helsper, H.; Allmaier, G.; Kammer, F. V.; Ramsch, R.; Solans, C.; Tomaniová, M.; Hajslova, J.; Weigel, S. Identification and Characterization of Organic Nanoparticles in Food. *TrAC Trends in Analytical Chemistry* **2011**, *30* (1), 100–112.

5. Krishnamoorti, R. Technology Tomorrow: Extracting the Benefits of Nanotechnology for the Oil Industry. *Journal of Petroleum Technology* **2006**, *58* (11), 24–26.

6. Mohanraj VJ, Chen Y, (Eds): Nanoparticles. J Pharmaceutical Research. **2006**, 5: 561-573.

7. Liou, S. About Free Radical Damage. https://hopes.stanford.edu/about-free-radical-damage/ (accessed Oct 10, 2019).

8. Milman U, Blum S, Shapira C, et al. Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2

diabetes mellitus and the haptoglobin 2-2 genotype. A prospective double-blinded clinical trial. *Arterioscler Thromb Vasc Biol.* **2007**:ATVBAHA.107.153965.

9. Bartlett HE, Eperjesi F. Effect of lutein and antioxidant dietary supplementation on contrast sensitivity in age-related macular disease: a randomized controlled trial. Eur J Clin Nutr. **2007**; 61:1121-27.

10. Burits, M. and Bucar, F., Antioxidant activity of *Nigella sativa* essential oil. Phytother. **2000**. Res., 14: 323-328. doi:10.1002/1099-1573(200008)14:5<323::AID-PTR621>3.0.CO;2-Q

11. G. Corona, M. Deiana, A. Incani, D. Vauzour, M. A. Dessi, J. P. E. Spencer, Mol. Nutr. Food Res. **2009**, 53, 897–903;

12. S. Singla, P. Karam, A. J. Deshmukh, J. Mehta, H. Paydak, J. Cardiovasc. Pharmacol. Ther. **2012**, 17, 12–20.

13. Rodriguez-Morato J., Xicota L., Fito M., Farre M., Dierssen M., de la Torre R. Potential Role of Olive Oil Phenolic Compounds in the Prevention of Neurodegenerative Diseases. Molecules. **2015** ;20:4655–4680. doi: 10.3390/molecules20034655.

14. Parkinson L., Cicerale S. The Health Benefiting Mechanisms of Virgin Olive Oil Phenolic Compounds. Molecules. **2016** ;21:1734. doi: 10.3390/molecules21121734.

15. Cicerale S., Lucas L., Keast R. Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. Curr. Opin. Biotech. **2012** ;23:129–135. doi: 10.1016/j.copbio.2011.09.006.

16. Wani T.A., Masoodi F.A., Gani A., Baba W.N., Rahmanian N., Akhter R., Wani I.A., Ahmad M. Olive oil and its principal bioactive compound: Hydroxytyrosol—A review of the recent literature. Trends Food Sci. Technol. **2018** ;77:77–90. doi: 10.1016/j.tifs.2018.05.001.

17. Marković, A. K.; Torić, J.; Barbarić, M.; Brala, C. J. Hydroxytyrosol, Tyrosol and Derivatives and Their Potential Effects on Human Health. *Molecules* **2019**, *24* (10), 2001.

18. Vshivkov, S.; Soliman, T.; Kluzhin, E.; Kapitanov, A. Structure of Poly(Acrylic Acid), Poly(Methacrylic Acid) and Gelatin Solutions. *Journal of Molecular Liquids* **2019**, *294*, 111551.

19. V.P. Belousov, M.Yu. Panov, Thermodynamics of Nonelectrolyte Aqueous Solutions, Khimiya, Leningrad, **1983**.

20. Tamang, S.; Reiss, P.; Texier-Nogues, I.; Labbé Pierre. Synthesis and functionalisation of near infrared emitting nanocrystals for biological imaging. thesis, **2011**.

21. IR Spectrum Table & Chart. https://www.sigmaaldrich.com/technical-documents/articles/biology/ir-spectrum-table.html (accessed Apr 5, 2020).