5-16-2022

The Use of Silica Nanoparticles for Controlled Drug Delivery of Nicotine

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An Honors thesis submitted in partial fulfillment of the requirements for Honors in Chemistry

By Jennifer Iwenofu

Under the mentorship of Dr. Ji Wu

Abstract

Drug molecules can be administered in different ways to the human body. Nanotechnology stands out over other alternatives by delivering drugs to specific locations and reducing reactivity time. In this research, the hydrogen bond between silica nanoparticles and the drug molecule, nicotine, were studied to controllably deliver nicotine by varying pH values.

_____ Thesis Mentor: _____

Dr. Ji Wu

Honors Director: _____

_____Dr. Steven Engel

April 2022

Department of Chemistry and Biochemistry

Honors College

Georgia Southern University

Acknowledgements

My wonderful, patient and brilliant mentor, Dr Ji Wu

The Honors College

Charity Femi-Oyetoro

Jake Dicesare

Department of Chemistry and Biochemistry

Table of Contents

Introduction

Drug delivery is a system of delivering pharmaceutical substances to produce an enhanced treatment efficacy in humans and animals. There are various forms of administering drugs into the body. Oral delivery utilizes capsules and pills. Intravenous delivery implements the use of injection. Pulmonary and Nasal delivery systems utilize aerosols and sprays. With regular drug molecules, specification and solubility is limited. To produce the necessary therapeutic benefits for the treatment of diseases, patients must take high doses of the medication. To address these issues, pharmaceuticals include a variety of drug carriers that can be used to distribute therapeutic agents to the desired site in the body.[1] Delivering drugs this way aims for a more targeted approach to the location of the body that needs the drug.

A controlled drug delivery method enables a predetermined way of drug release and drug degradation after its available period for use. This method reduces side effects of drugs, improves aqueous solubility and chemical stability of the drug agents.[2] It is a specific stimulus designed for the drug delivery that effectively controls the distribution. For example, liposomal doxorubicin, a liposome drug, has been approved by the U. S. Food and Drug Administration (FDA) as an effective process that improves bioavailability and efficacy.[2]

Nanotechnology has been used in various areas in the past. In recent times, it has been developed to be used in medicine. Nanoparticles have been used for continuous practice of creating more effective delivery of various drugs. They have a porous structure with a broad surface area that enables the attachment of various functional groups to target the drug moiety to a specific site. Examples of nanotechnology include nanogels, coated nanoparticles, pegylated nanoparticles, metal nanoparticles and solid lipid nanoparticles. Silica nanoparticle is an example of an inorganic

nanoparticle. Mesoporous nanoparticles are spherical in shape. Meanwhile, some other nanoparticle shapes can be rod-like, crystalline, or cubic. These Silica nanoparticles have a high drug loading capacity and low toxicity which makes them suitable for controlled drug delivery. Because of the strong Si-O bond, they are more stable to degradation and stress in comparison to liposomes.[3]

In this research, drug molecules like nicotine are incorporated into silica nanoparticles through a tunable and reversive H-bonding and then controllably released by adjusting pH to break up the H-bond. Parameters such as pH and pKa are being varied to study their effect on the self-assembly and breakdown of silica nanoparticles-drug molecules matrix. The existence of hydrogen bonding is proved by using the Fourier-transform infrared spectroscopy (FTIR). The rate of drug release is determined using Shimadzu High Performance Liquid Chromatography (HPLC). External standard methods are going to be developed, validated and optimized for the HPLC analyses. The results of this project will provide enlightened direction toward the future of controlled, cost effective, safe delivery of drugs.

Research Hypothesis and Objectives

Our hypothesis is that drug delivery rates of nicotine can be controlled by adjusting pH which can affect the self-assembly of functionalized silica nanoparticles and drug molecules based on H-bonding. To test the hypothesis, the following objectives are proposed: 1) to synthesize silica nanoparticles:

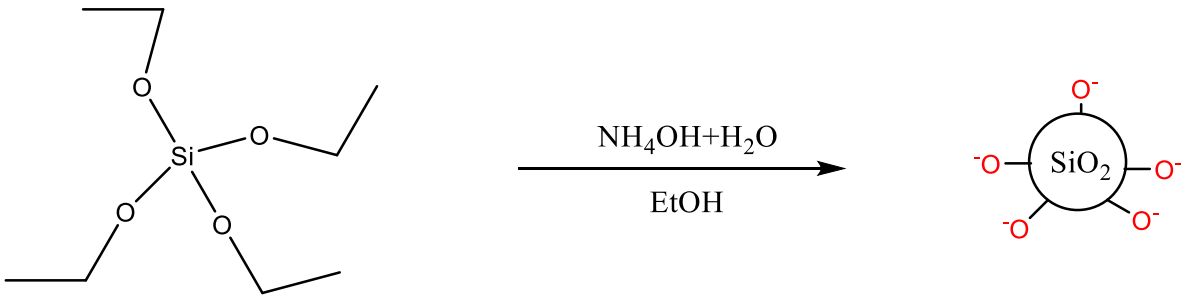

Figure 1- The synthesis of silica nanoparticles from Tetraethyl Orthosilicate (TEOS).

2) To optimize the self-assembly of silica nanoparticles and nicotine by adjusting pH, solvent, concentration and mass ratio between silica nanoparticles and the drug molecule:

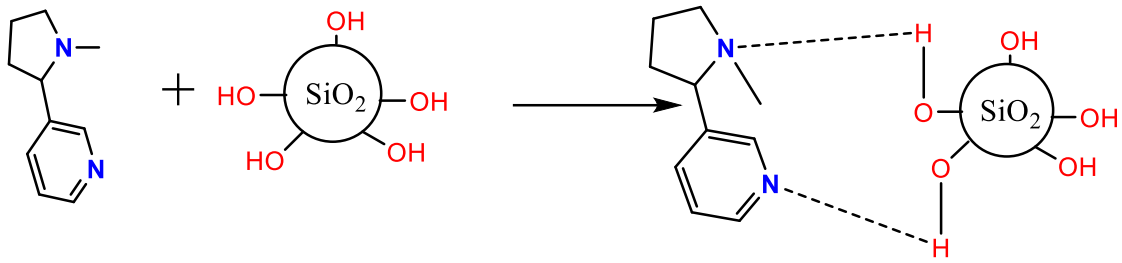

Figure 2- The incorporation of nicotine into silica nanoparticles.

3) To quantify the drug release rate from the silica-nicotine assembly at different pH, via HPLC analysis.

Experimental design

Synthesis of nanoparticles

To test the hypothesis, a series of procedures were conducted. The silica nanoparticles were synthesized using the Stöber method. This method involved reacting tetraethoxysilane (99% Alfa Aesar) in various alcoholic solutions and bases.[4] The alcohol we used was Pharmco ethanolACS grade and the base we used was ammonium hydroxide. The ammonium hydroxide was ACS Grade of 28-30% and was produced by VWR Analytical. Cetyltrimethylammonium bromide was not used as a base because it has to be removed by calcination and synthesizing nanoparticles without calculating the particles, increases drug loading on the nanoparticles.[5] For the 100 nm silica nanoparticles, the optimum synthesis time was 24 hours while that of the 50 nm was 14 hours. The amount of ethanol, ammonium hydroxide and deionized water varied depending on the desired size. The size was confirmed using Malvern Zetasizer Nano Series Nano-ZS Dynamic Light

Scattering (DLS). After synthesis of the nanoparticles, the particles were centrifuged to decant all the ethanol and left to dry overnight. After drying, the clear silica nanoparticle crystals were evident and grinded to become fine powder.

Incorporation of drug molecule

(S)-(-)-Nicotine (99% Alfa Aesar) was incorporated into the silica assembly. Various mass ratios of 1:1, 10:1, 20:1 have been used and the most effective is 10:1. One of the solvents used to incorporate the silica nanoparticle was fisher chemical tetrahydrofuran. 0.17grams of pure silica nanoparticles were measured and added to a conical flask containing 5 ml of tetrahydrofuran. 0.17ml of nicotine was added to the mixture. The solution mixture was left to dry overnight and after drying was washed with ethanol to remove any contaminating solvents. Another method used to incorporate the silica nanoparticles and the nicotine was using fisher chemical chloroform as a solvent. The same amount of silica nanoparticles and nicotine were added to the chloroform. They were left to stir for twenty minutes before drying at 40^{o}C for twenty-five minutes. The third method was adding 0.17ml and 0.17g of nicotine and silica nanoparticles in 5ml of chloroform and sonicating for 10 minutes. They were left to dry overnight. The fourth method was adding the same amounts of the silica and the nicotine in VWR Analytical ACS dichloromethane. Due to the volatile nature of dichloromethane the solution was left to stir for 20 minutes as the dichloromethane evaporated in an oven. The silica nicotine mixture was then filtered and washed using VWR chemicals HPLC grade diethyl ether, not stabilized. Diethyl ether was used to wash the mixture for the last three methods used. The different methods are summarized in table 1. The resulting powder was measured and analyzed using the Smart iTX Thermo Scientific Fourier-transform infrared spectroscopy (FTIR). After a series of tests, there was a more efficient method of incorporating the nicotine into the silica nanoparticles. Lastly, after silica nanoparticles were

centrifuged four times at 10,000RPM and the supernatant was removed, the pellet was washed with dichloromethane twice to remove any ethanol present. 1ml of Nicotine was added to the nanoparticle pellet, vortexed and left to dry in an oven overnight. When the mixture has dried, the crystals formed are grinded using a mortar and pestle.

Loading efficient test procedure

The loading efficient test procedure showed the amount of drug molecule loaded into the silica nanoparticle. Loading efficiency is the amount of nicotine (gram) loaded in 1 gram of silica nanoparticles. The last method used to incorporate silica nanoparticles and nicotine was used. After the compound was grinded, the crystals formed turned to fine powder. 0.17grams of the powder was added to 5ml of pH 1 and pH 10. The solution was placed in a 50ml beaker wrapped with aluminum foil and left to stir for two days. After two days 1ml of the solution was ejected using a syringe and filtered using a VWR 13mm Syringe Filter w/ 0.2 µm PTFE membrane. The filtered solution was transferred to a glass vial (VWR Convenience Kit 9-425 Clear Glass Blue Cap, PTFE/Silicone, Pre-Slit) and placed in the HPLC machine for analysis

High Liquid Performance Chromatography

The Shimadzu HPLC contains four components. The components include an LC-20AT liquid chromatography, an SIL-20A HT prominence autosampler, an SPD- 20A UV-Vis detector and a CBM-20A communication bus module. A Thermo Scientific HyPURITY C18 column (150 x 4 mm) was used as the stationary phase. The mobile phase with the highest elution profile was 0.1% Tetra ethylamine (99% fisher chemical assay) in HPLC grade water from fisher chemical. The UV-Vis absorbance detector wavelength was set at 254 nm and the flowrate was 1ml/min.

To find the elution time of nicotine in the HPLC, a nicotine standard was run. 100ppm stock of nicotine standard was made by adding 10 microliters of nicotine into 100 milliliters of deionized water. Various ppm concentrations were made from Nicotine stock.

To characterize the data, JEOL JSM-7600F Field Emission Scanning Electron Microscope coupled with the Transmission electron microscopy method (TED), Direct Light Scattering (DLS), were used to investigate the morphology, and size. Fourier transform infrared spectroscopy was used to prove the incorporation of nicotine into the silica nanoparticles.

Diffusion study

The diffusion study of nicotine was conducted using a 5mm Franz cell. 0.01g of the drug infused into the silica nanoparticles was added to a Franz cell. The pH studied were 1M pH 1 hydrochloric acid buffer, 0.01M pH 6 Phosphate buffer and 0.01M pH 10 Carbonate-Bicarbonate buffer. Using a syringe 5ml of each pH buffer was added to the Franz cell. A magnetic stir bar was added to mix the compound and buffer solution. After 1 hour another syringe was used to collect 1ml from the Franz cell. The mixture was filtered using the PTFE membrane filter and transferred to glass vial for HPLC to determine the concentration of nicotine released after diffusion study. The Franz cell has proven to be a good in vitro model for processes that will happen in the body.[6]

Results

Table 1- Summary of the different experimental designs employed to incorporate silica nanoparticles and nicotine.

Method	**1**	**2**	**3**	**4**	**5**
Solvent	Tetrahydrofuran	Chloroform	Chloroform	Dichloromethane	Dichloromethane
Washing solvent	Ethanol	Ethanol	Ethanol	Diethyl ether	Diethyl ether
Nanoparticles: Nicotine ratio	1:01	1:01	1:01	1:20	1:10
Reaction time	N/A	25 minutes	10 minutes	20 minutes	N/A

Table 2 -The IR analysis of silica nanoparticles, pure nicotine and nicotine incorporated into silica nanoparticles.

Compound	**Stretch (cm^{-1})**
Pure Nicotine	C-H stretch - 3029 (alkene)
	C-H stretch - 2967, 2942, 2773
	C=N - 1691
	Methyl group- 1456
	C-N stretch of aromatic amine - 1314
Silica nanoparticle	O-H stretch vibration - 3232
	Asymmetric vibration of Si-O stretch- 1061
	Si-OH stretch- 951.2 asymmetric vibration of OH
Silica and Nicotine	O-H stretch- 3213
	C-H stretch - 2970, 2780
	C=N stretch- 1650
	C-H bending- 1477
	C-N stretch of aromatic amine- 1316
	Si-O stretch -1039
	Si-OH stretch - 954.3

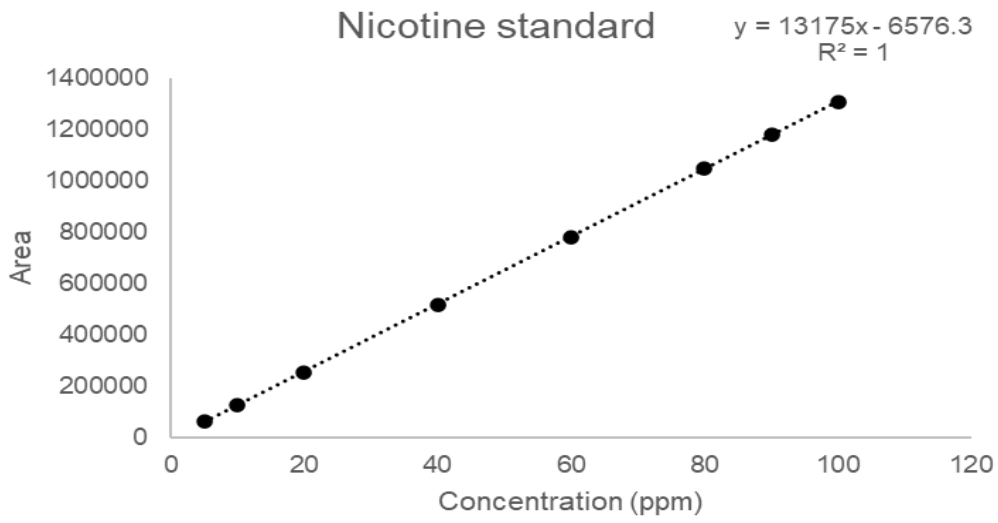

Figure 3- The external standard calibration curve of nicotine used to determine nicotine delivery rates.

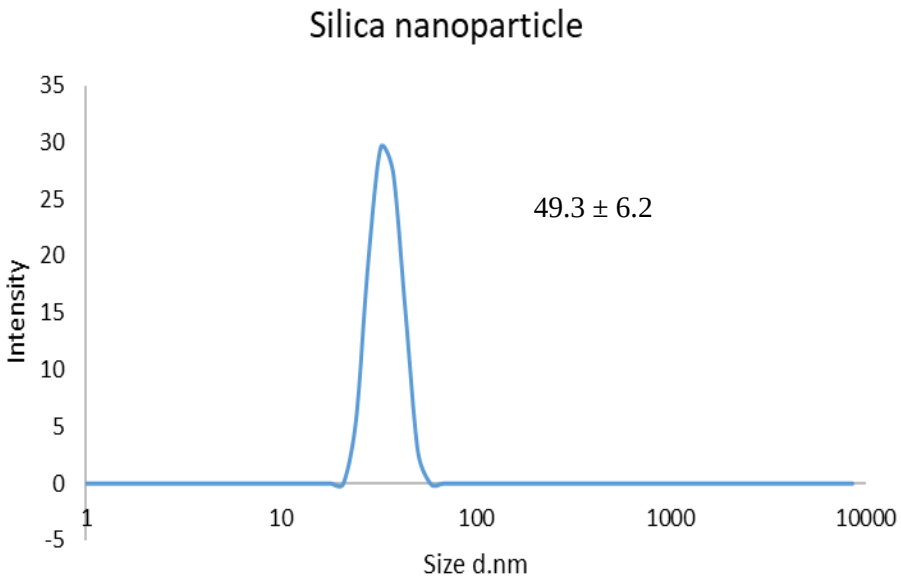

Figure 4- DLS spectrum of SNPs with an average diameter of ~50 nm.

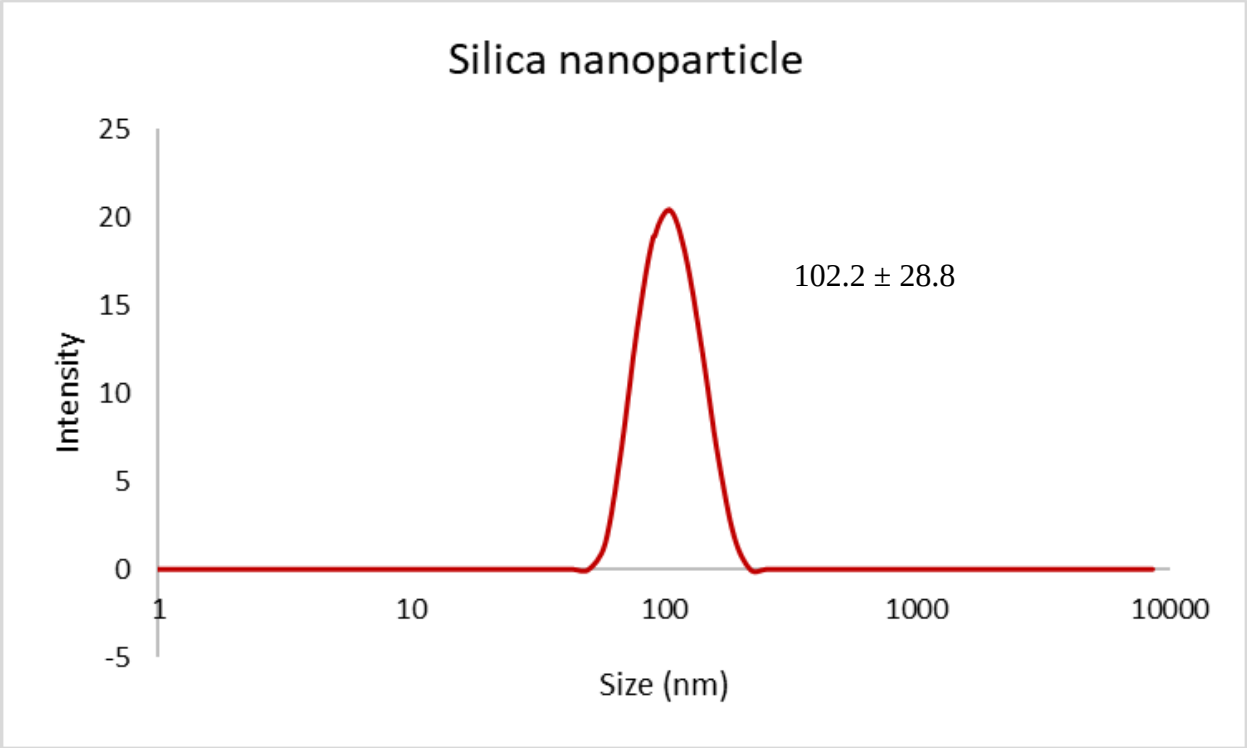

Figure 5- DLS spectrum of SNPs with an average diameter of ~100 nm.

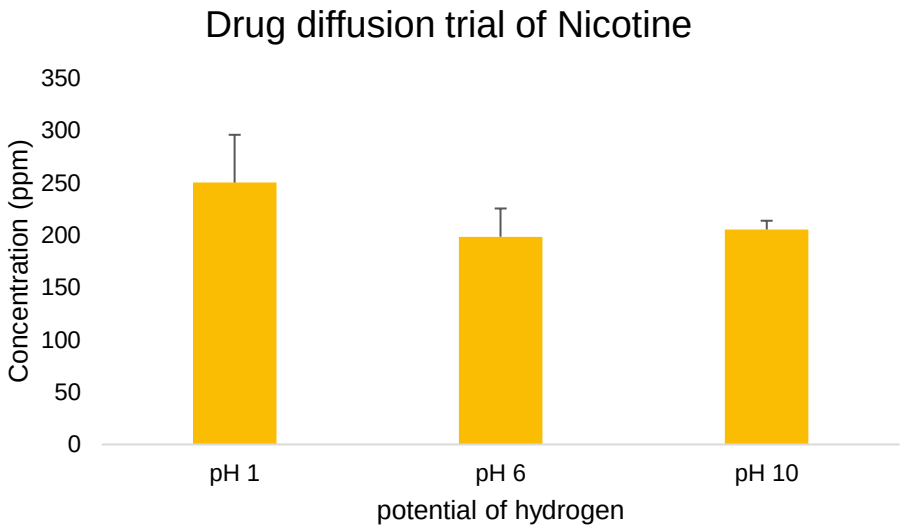

Figure 6- Drug diffusion study of nicotine at pH 1, pH 6, pH 10

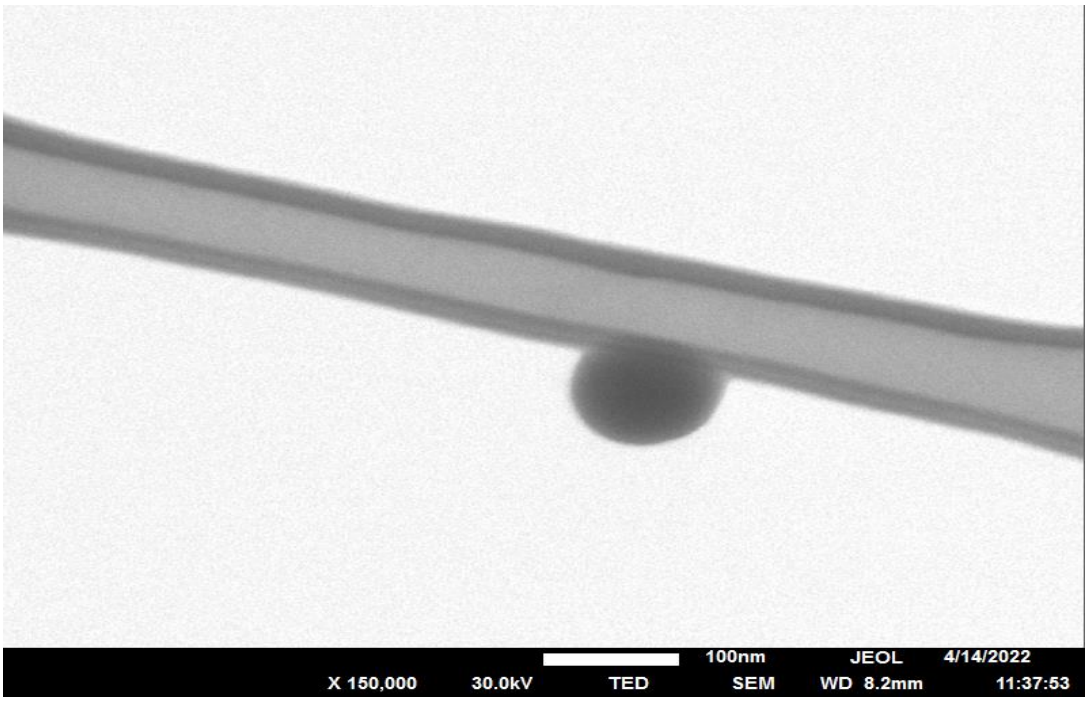

Figure 7- TEM image of one 50 nm synthesized silica nanoparticle

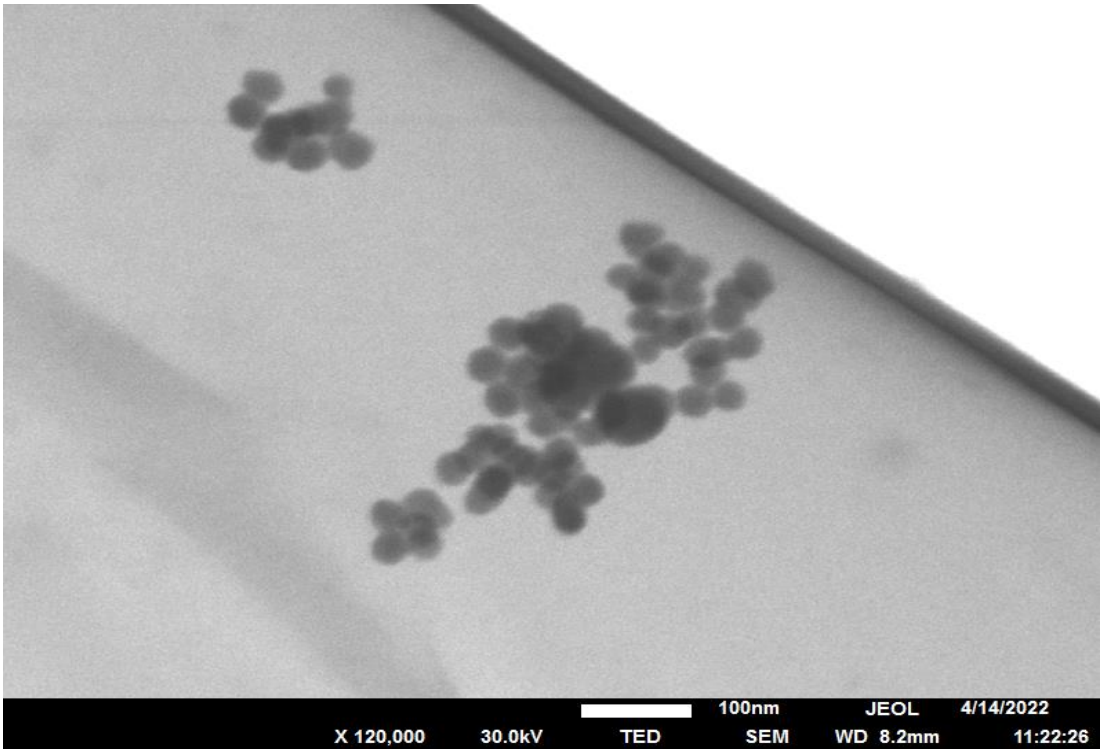

Figure 8- TEM image of a cluster of synthesized 50 nm silica nanoparticles.

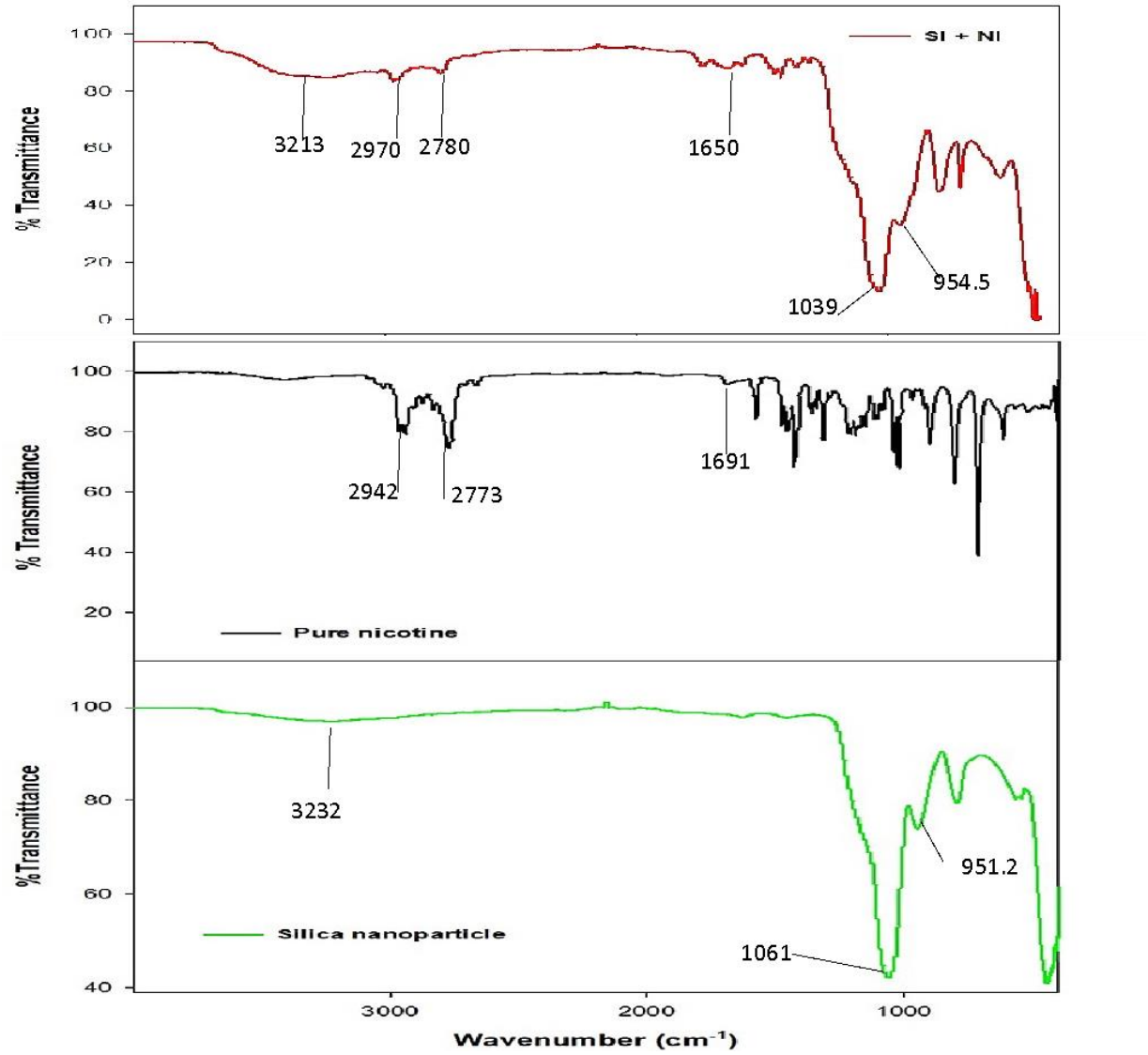

Figure 9- FTIR spectra for nicotine incorporated in silica, pure nicotine, and pure silica nanoparticle with peaks detected.

Discussion

The silica nanoparticles were produced successfully using the Strober method. Initially, this process was regulated based on time and amount of ethanol as well as ammonium hydroxide. As more experiments were done, it was understood that the order in which the chemicals were added

affected the rate of reaction. When ammonium hydroxide, which is the catalyst, was added, the reaction occurred at a much faster rate. The different sizes were found using the Dynamic Light Scattering spectroscopy. Figures 4 and 5 show the average size of nanoparticles and standard deviation after the synthesis and predicted time. The Transmission Electron Microscopy shows the structure of the silica nanoparticles. As seen in the figure 7 and 8, the silica nanoparticles have a diameter of 50 nm. This TEM image visualizes the morphology of the nanoparticles. Based on this result, it can be seen that the silica nanoparticles are indeed spherical in shape. The nanoparticles are spherical in shape and the Nicotine molecules can attach on the surface of the nanoparticles. The silica nanoparticles incorporated with nicotine immediately after the centrifugation process was the most efficient procedure. This process enabled a sufficient amount of nicotine to be loaded into the nanoparticles. The loading efficiency of the nanoparticle and nicotine mixture was used to find how much nicotine was loaded in the nanoparticle. After HPLC analysis of the loading test, the concentration of both pH 1 and 10 was found using the standard equation. Due to the standard concentration in ppm, this final concentration was also in ppm. The concentration was converted to mg/L using the amount of the silica and nicotine solution (0.005L) that was analyzed in the HPLC. This was the actual mass of nicotine based on the HPLC analysis which was 1.84mg from pH 1 and 1.68mg from pH 10. The surface area of silica nanoparticles can be found using $\frac{6000}{\left(density\ in\ \frac{g}{cm^3}\right)*diameter}$[7]. The density of silica nanoparticles is 2.65g/cm^3.[8] The diameter of the nanoparticle synthesized was 60nm. This equation gave 13.81m^2/g surface area. This means that on each nanoparticle it has a surface area of 13.81m^2/g in one gram of the particles. 0.089grams of the nanoparticles were used, therefore the calculation was adjusted using the equation below to 1.23m^2/g in 0.089grams of the silica nanoparticles. $grams\ in\ \frac{m^2}{g} = \frac{m^2}{g} * grams\ of\ nanoparticle\ used$. On each silica particle there

are 4.9OH groups around the atom as seen in figure 1. To find how many hydrogens bonds that could have formed, the surface area was converted to nm^2 using the amount of OH groups found on the surface. $surface\ area\ in\ \frac{m^2}{g} * 10^{18} * 4.9 =$ $hydrogen\ bond\ present\ on\ each\ surface$. This value was converted to moles using Avogadro's number using the equation: $hydrogen\ bond\ present\ on\ each\ surface*$ Avogadro's number = moles. The moles were converted to grams of nicotine using nicotine's molar mass to be 4.44mg. $mass = mole * molar\ mass$. The yield of this loading was 41.3% meaning almost half of the silica nanoparticles and the nicotine were incorporated into each other. This low yield could be due to steric hindrance of the bulky nicotine molecule. The drug delivery test in figure 6 shows the different drug release in concentration based on the pH. The pKa's of nicotine are 3.2 and 7.9[9]. That means below pH 3.2, both hydrogens on the amines in the nicotine are protonated. Once above pH 3.2, one of the amines has donated hydrogen. Higher than pH 7.9, both amines have donated their hydrogen. The silanol group on the silica nanoparticle has a pKa of 8.5.[10] When pH is lower than 3, both the nicotine and the silica are protonated, therefore hydrogen bonds will be weakened, and there will be high drug release rate. When pH is higher than 8, both the silica nanoparticle and nicotine are deprotonated, therefore the hydrogen bond will also be weak resulting in fast drug release. When pH is in the middle range, between 4,5, and 6, one amine group is protonated while the other is not. The silanol group is deprotonated, and one of the amines in nicotine is protonated, thus slowing down the release of nicotine due to the effect of H-bonding. As seen in figure 6, when the pH is 1, there is a higher release of the nicotine from the silica nanoparticle. This pH shows the largest release rate of the nicotine molecule. This is consistent with the hypothesis because both the silanol and the nicotine are protonated. At pH 6, there is a high drug release rate of nicotine. At pH 10, the

rate of drug release is slightly higher than that of pH6. The standard deviation of this pH is lower, meaning the data is more reliable. While it is expected for the drug release rate to be much lower in pH6, it might be higher because the hydrogen bonds are very weak and easily broken. The drug diffusion study in a Franz cell mirrors that of the human body. In the stomach the pH is much lower due to release of HCl. At this low pH, the drug can be loaded into the stomach at higher amounts. In the duodenum, bicarbonate is introduced to increase pH. At this site, the drug delivery rate will be lower because of the higher pH. This enables us to regulate the exact site the drug should be released. The hydrogen bond was proved using the FTIR machine. As seen in table 2 and figure 9 the IR spectra of the silica and nicotine shows a combination of both the pure silica spectra and the pure nicotine spectra. There are some distinct peaks found in each spectrum that can be seen on the mixture spectra. For example, in pure nicotine, there is a C=N stretch through the vibration of carbon and nitrogen at 1691cm^{-1} and the nicotine and silica mixture this peak is at 1650cm^{-1}.[11] When new hydrogen bonds arise, it weakens the previously existing bond it binds to. Hydrogen bonds break up the energy involved during stretching. Wavenumber is directly proportional to energy so when energy reduces, the wavenumber reduces. In comparison to the silica nanoparticle, the OH group on the silica is seen at 3232cm^{-1} while on the silica and nicotine spectra that peak is at 3213cm^{-1}. [11] There is also a reduction in the previous hydrogen bond of the silanol group. The bond is based on the oxygen and hydrogen stretch vibration.

Conclusion and Future Work

Nicotine, a drug molecule, has two amine groups that can undergo a hydrogen bond in a suitable environment with silica nanoparticles whose surface is attached with silanol groups as verified by FTIR. The organic drug molecule and the inorganic molecule formed hydrogen bonds that can be broken depending on the pH, which fact has been confirmed by our HPLC analysis.

The hydrogen bond forming, and bond breaking may be used to regulate and control the shrug delivery of nicotine into the human body in our future studies.

In future, there are other steps that can be incorporated to understand this drug delivery method. In this research, only three pH's were tested. Other pH values can be tested as well to test the drug release over a wide range of pH. Silanol has different pKa values which affects the hydrogen bond hypothesis. If the pKa value varies, the drug release might be variable at different pH values.

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Acknowledgements:

I would like to thank my research mentor, Dr Ji Wu. None of this would have been possible without his help and guidance. I would like to thank the honors college for this great experience. It has exposed me to new knowledge I would not have gained otherwise. I would like to thank Charity Femi-Oyetoro. She taught me a lot about this project and trained me on so many techniques. Thank you, Logan Williams, for taking time out of your day to help me run the TEM data for this project. Thank you, Emmanuel Fasusi, for teaching me how NMR works and helping me plot my data. Thank you, Jake Dicesare, for helping me run an SEM for the silica nanoparticle. Thank you, Katie Nolan, for explaining things when the concepts were hard for me to grasp. Thank you, Dr Abid Shaikh, for giving me dichloromethane to use when my lab ran out of the chemical. Thank you, department of chemistry and biochemistry. So many professors helped in many ways on this project. Thank you for keeping the doors open for us students to expand our minds. Thank you for constant encouragement and feedback.