EMPLOYING CHROMATOGRAPHIC AND SPECTROSCOPIC METHODOLOGIES TO MONITOR DNA RESPONSE TO PLATINUM-BASED CHEMOTHERAPEUTIC DRUGS

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF CHEMISTRY

IN THE GRADUATE SCHOOL OF THE

TEXAS WOMAN'S UNIVERSITY

DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

COLLEGE OF ARTS AND SCIENCES

BY

SKYLAR C. WAPPES, B.S.

DENTON, TX

AUGUST 2020

Copyright© 2020 by Skylar C. Wappes

DEDICATION

I dedicate this thesis to my Mom, Dad, family, and friends who loved and supported me through this journey. I also dedicate this thesis to any young girls out there dreaming of becoming a woman in STEM.

ACKNOWLEDGEMENTS

My deepest and sincerest thank you to my mentor, Dr. Nasrin Mirsaleh-Kohan for giving me the opportunity to be a part of her research group as an undergraduate and encouraged me to pursue my Master's – I would not be where I am today if it wasn't for her guidance and support.

I want to also give a huge thank you to the chair of the Department of Chemistry and Biochemistry here at TWU – Dr. Richard Sheardy. Dr. Sheardy has been a wonderful advisor and mentor who helped shape me into the woman scientist I am today.

I also want to thank Dr. Robby Petros, Dr. Yunxiang Li, and Dr. John Beatty for their support and help throughout my research. Thank you to my lab partners—Trang Nguyen, Yesenia Olguin, Jessica Hernandez, and Catherine Perez.

Most importantly, I want to thank all professors and teachers I have had throughout my academic career who gave me the knowledge and drive to pursue my dreams.

ABSTRACT

SKYLAR C. WAPPES

EMPLOYING CHROMATOGRAPHIC AND SPECTROSCOPIC METHODOLOGIES TO MONITOR DNA RESPONSE TO PLATINUM-BASED CHEMOTHERAPEUTIC DRUGS

AUGUST 2020

In order to design platinum-based chemotherapeutic drugs that express low toxicity and high efficiency, their nature of interaction with DNA, effectiveness, as well as their mechanisms need to be better understood. This research investigates how cisplatin, cis-diamminedichloroplatinum (II), and its derivatives, carboplatin, nedaplatin, and oxaliplatin, effect DNA stability and structure. Two sensitive and selective analytical methodologies—Surface-Enhanced Raman Scattering (SERS) and High-Performance Liquid Chromatography (HPLC)—were applied to monitor the key concepts stated above. In this project, it was found that of all the drugs, carboplatin did not significantly modify the DNA. Even though carboplatin complexes were made at a 1:2 volume ratio it left more DNA unbound (5% unbound) compared to the other drug complexes which were ran at a 1:1 ratio (<2% unbound). Averaging the drug interactions, cisplatin and oxaliplatin left less DNA unbound than nedaplatin did, but carboplatin in all HPLC experiments left significantly more DNA unbound. In both SERS and HPLC experiments, carboplatin modified each DNA less than cisplatin.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Background

The implementation of platinum for chemotherapeutic purposes has been deemed effective and efficient for treating aggressive cancers such as testicular, lung, ovarian, and breast. Cisplatin, cis-diamminedichloroplatinum (II), is the first-generation platinum-based chemotherapeutic drug coined by Rosenburg et al at Michigan State University.[25] Cisplatin is commonly used to treat testicular and ovarian cancer. It is known to initiate cell death by directly binding to DNA and deprive it of replication, ultimately activating several signal pathways.[1] Cisplatin is highly effective, but consequently very toxic. Since the discovery of cisplatin, many cisplatin derivatives have been synthesized, but few ever make it to clinical trial.

Carboplatin, cis-diamine (1,1-cyclobutanedicarboxylato) platinum (II), is a cisplatin derivative and second-generation platinum-based chemotherapeutic drug, but while being less toxic, carboplatin is also less effective. Carboplatin is known to treat head,

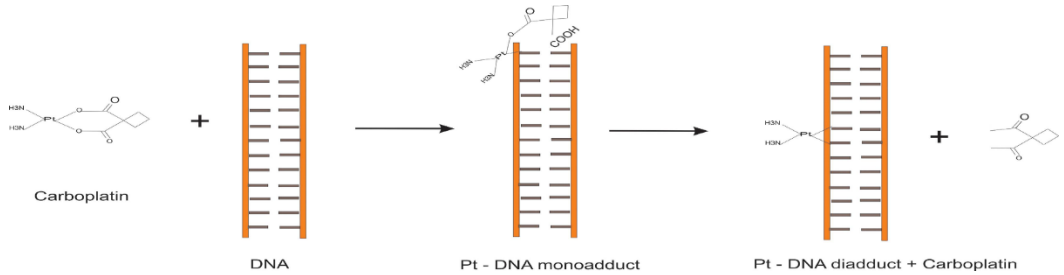


Figure 1. Illustrates the mechanism of carboplatin interacting with DNA.[11]

neck, testicular, and bladder cancer. Limitations have also set in for its chemotherapeutic effect due to recent discoveries of carboplatin-resistant tumor cells.[2] **Figure 1** shows the mechanism for carboplatin.

Nedaplatin, cis-diammine(glycolato)platinum II, is another cisplatin derivative and second-generation platinum-based chemotherapeutic drug. It is known to bind to nucleophilic groups of DNA.[3] nedaplatin has succeeded in being less nephrotoxic and neurotoxic than both cisplatin and carboplatin. nedaplatin was mainly developed to decrease the toxicities caused by cisplatin.[4] More recent third-generation derivatives have been synthesized, such as oxaliplatin and heptaplatin. Oxaliplatin (Eloxatin), Oxalato(1,2-diaminocyclohexane)platinum(II) is primarily used to treat colon cancer. The mechanism for oxaliplatin varies slightly from the other drugs mentioned, in that it consists of displacement of the oxalate group by H_2O and Cl^- inducing its anticancer activity. Heptaplatin, cis-malonato(4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane) platinum (II), has been found to treat cancers that have inherited resistance to cisplatin.[6]

Structurally, platinum-based chemotherapy drugs are known to have a single central platinum atom surrounded by two ammine groups (bidentate 1,2-diaminocyclohexane for oxaliplatin) and two cis ligands (see **Figure 2** below). These cis ligands can be further characterized as leaving groups due to their departing from the platinum core. Once these ligands are detached, they are replaced with aquo groups (with the exception of oxaliplatin, as mentioned above) ultimately forming an anticancer-active metabolite: diaquo-diamine-platinum.[7] The diaquo-diamine-platinum complexes react

with amine groups of DNA preferably binding covalently at the N-7 terminal of the purine bases guanine and adenine leading to platinum-DNA adducts. This interaction forms intrastrand crosslinks between bases in the same strand or interstrand crosslink between bases in opposite strands.[7] Therefore, when the detached ligands are replaced with aquo groups (H_2O and Cl^- for oxaliplatin) the drugs anticancer mechanism is activated leading to the deprivation of replication on the DNA by the drug resulting in apoptosis, or cell death.

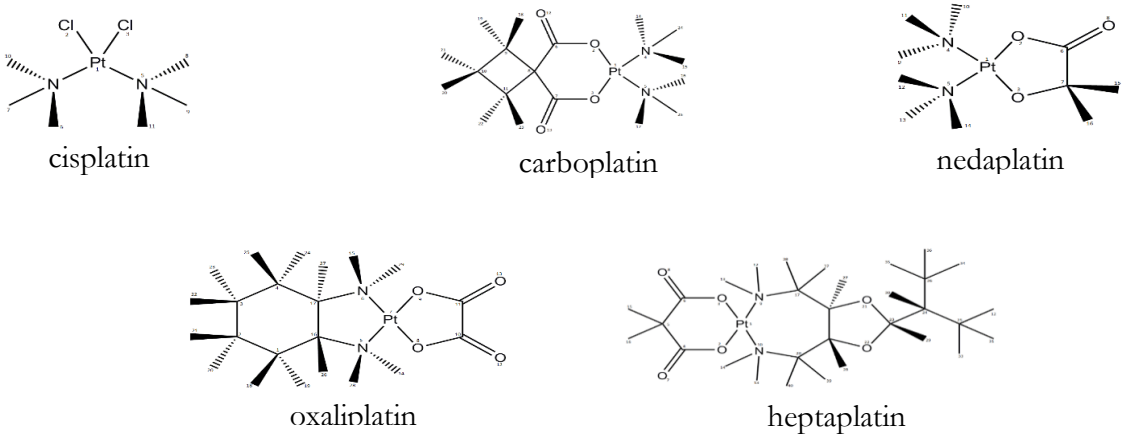


Figure 2. Structures of platinum-based chemotherapeutic drugs: cisplatin, carboplatin, nedaplatin, oxaliplatin, and heptaplatin.

CHAPTER II

EXPERIMENTAL TECHNIQUES

Spectroscopic Technique: Surface-Enhanced Raman Scattering

Surface-enhanced Raman scattering (SERS) is a highly sensitive spectroscopic method which measures vibrational modes of compounds and molecules.[8] Molecules are exposed to light radiation at a certain frequency, the radiation is then scattered from the molecule causing wavelength shifts referred to as stokes shift and anti-stokes shift. Both shifts can be calculated using the equation:

$$E = h\nu \pm \Delta E$$

Where E is energy, h is Plank's constant, ν is wavenumber, and ΔE is the change in energy from ground state to excited state.[23] **Figure 3** shows each component of a Raman instrument.

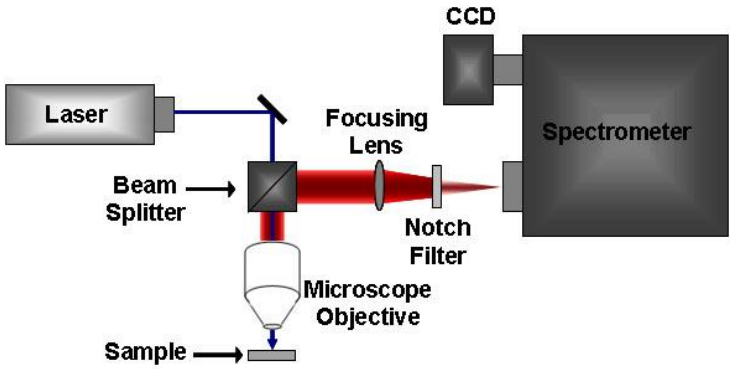


Figure 3. Illustrates the fundamental components of Surface-Enhanced Raman Scattering (SERS)[17]

Raman scattering exists as two characteristics: Rayleigh, which is more than 99% of scattered light radiation, and Raman, which is less than 1% of scattered light radiation. **Figure 4** illustrates both Rayleigh and Raman light scattering. Both Raman and Rayleigh scattering consists of photons and molecules colliding, but with Rayleigh the collision results in no net change in energy of the incoming light whereas with Raman the collision results in a net change in energy.[23] **Figure 5** shows the energy transitions of Raman and Rayleigh light scattering.

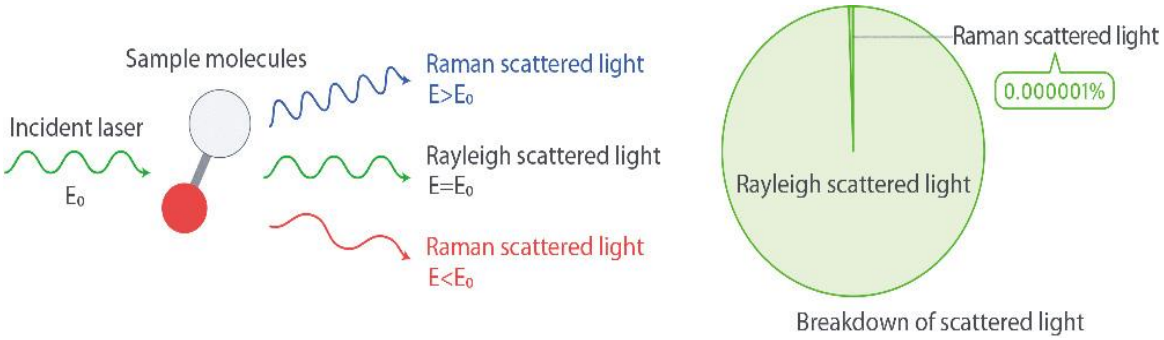


Figure 5. Illustrates Raman and Rayleigh light scattering in Raman Spectroscopy.[24]

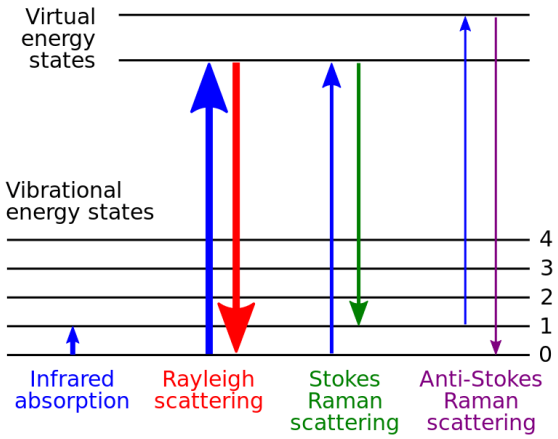


Figure 4. Represents the difference in energy for IR absorption, Rayleigh, Stokes, and anti-stokes Raman scattering.[24]

Raman Spectroscopy and Infrared Spectroscopy are complimentary to one another. In some cases, molecular vibrations are both IR and Raman active. Molecules can be both IR and Raman active if the consist of an inversion center. The Raman frequency shift and the IR absorption peak frequency are identical.[23]

The signal of Raman scattering is strengthened when the analyte is adsorbed on or near a metal surface such as silver nanoparticles.[9] The silver nanoparticles are synthesized using the popular Lee and Meisel technique.[10] Since Raman scattering results in less than 1% of the diffracted laser light being detected, the use of metal nanoparticles aid in increasing the Raman intensity and in some cases it will quench and fluorescence. The rigid structure of the silver nanoparticles enables the ability to not only focus on the interaction, but to allow more laser light to get diffracted and detected.

When metallic nanoparticles are excited by electromagnetic radiation, their conduction electrons exhibit a phenomenon known as localized surface plasmon resonance (LSPR), which depends on the size, shape, and dielectric environment.[15] Silver and gold nanoparticles both have LSPRs that cover the visible and near infrared wavelength range, therefore suitable for the Raman measurements. **Figure 6** below illustrates the difference in light scattering with and without metallic nanomaterials.

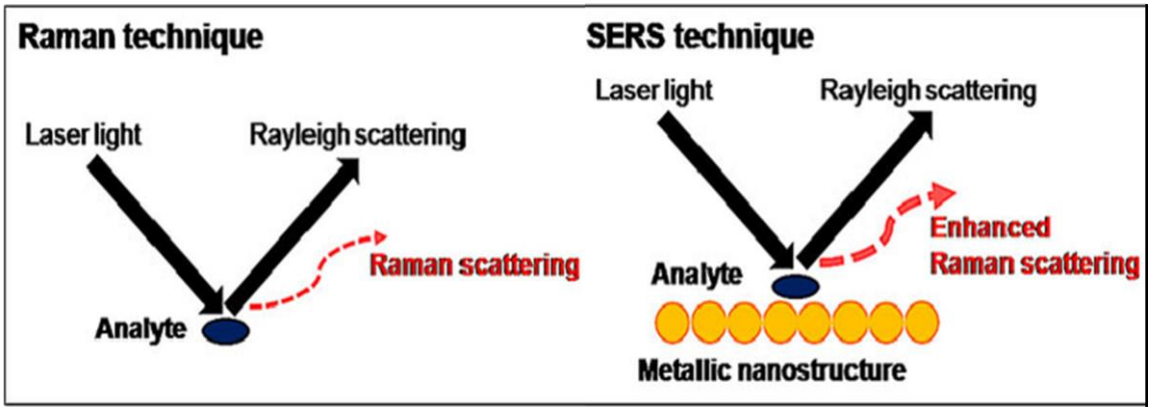


Figure 6. Illustrates the comparison of Raman Spectroscopy (left) and Surface-Enhanced Raman Scattering (SERS) (right).[18]

Chromatographic Technique: Reverse-Phase High-Performance Liquid Chromatography

High-Performance Liquid Chromatography (HPLC) is an analytical method used to separate nonvolatile or thermally unstable compounds (**Figure 7**). In this research, reverse-phase high-performance liquid chromatography (RP-HPLC) is used. RP-HPLC is a form of partition chromatography where the stationary phase (column) is relatively nonpolar and the mobile phase is polar. Having a nonpolar stationary phase and a polar mobile phase the more polar the solute is, the shorter the retention time will be. **Figure 8** is a diagram that shows the inside of the most common type of column (C_{18}) used in RP-HPLC which is also the column used in this research.

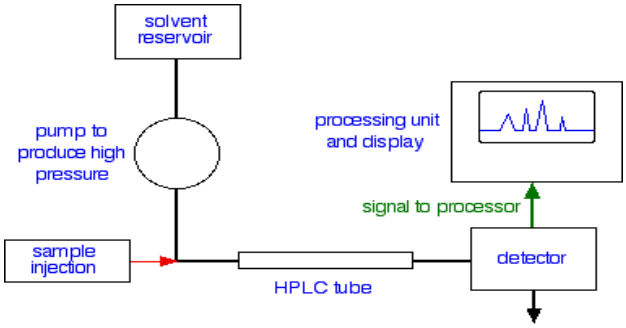


Figure 7. Illustrates the fundamental components of High-Performance Liquid Chromatography (HPLC).[16]

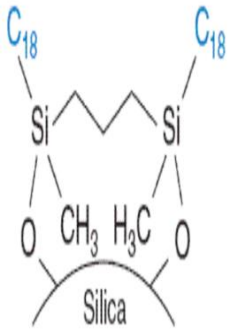


Figure 8. Illustrates the components inside a C_{18} column which was used throughout the HPLC experiments.[23]

By employing HPLC, components of both DNA and platinum drugs were separated and detected. HPLC, like SERS, allows for smaller sample volumes and lower concentrations. This method also produces data during smaller reaction times (less than 60min), Since DNA and the platinum drugs used throughout the experiment are polar compounds, polar solvents, Triethylammonium Acetate (TEAA) and H_2O were used to ensure proper separation occurred. The data produced from the HPLC experiments indicate if intermediates are produced from the interaction (most common intermediates are monochloro- or dichloro–diamine–platinum[14]), this allows for the determination of platination rates and percent concentrations. By observing the separated polar compounds

of each substance enables further research and better understanding of the interaction between the drug and DNA.

Several factors are monitored when analyzing data output from HPLC: qualitative and quantitative analyses, the capacity factor, and the peak resolution. Qualitative analysis primarily relies on the retention times whereas quantitative analysis relies on the peak area values. The capacity factor (*k*) is the measuring of the retention of a peak that is independent of the column and mobile phase. The capacity factor should be at least 1.0 for the first peak to ensure it is separated from the mobile phase but should not exceed 10-15 because the analysis time would be too long. The capacity factor can be calculated using the equations below t'_R represents the time the solute is eluted by the stationary phase and t_M represents the time it spends in the mobile phase[27]:

$$k = \frac{t'_R}{t_M} = \frac{t_R - t_M}{t_M}$$

Peak resolution (*R*) is the ratio of the distance between the two peak maxima (Δt) to the average value of the peak width at the base (w_b). The peak resolution primarily represents the efficiency of the column. The peak resolution can be calculated using the following equation[27]:

$$R = \frac{\Delta t}{w_{b^1} + w_{b^2}} = \frac{2\Delta t}{w_{b^1} + w_{b^2}}$$

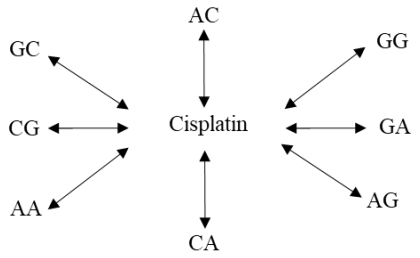
Drug to DNA Interaction

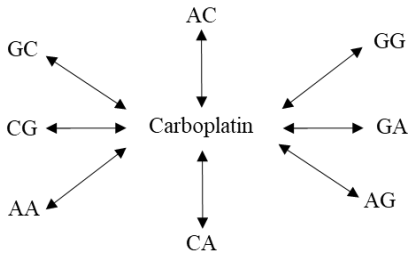
It is known that platinum drugs preferably interact with guanine over any other DNA base.[12,13] In a study conducted by Sheardy and coworkers looking at binding specificity of Co(III) with DNA, cisplatin preferentially interacts at GG sites predominantly through intrastrand crosslinks, at GA sites through intrastrand crosslinks, but to a lesser extent than GG sites, and at GC sites via interstrand crosslinks.[14] Because of this, the dinucleotides containing G and A sites were used throughout this research. The preferred binding to dinucleotides by platinum-based chemotherapeutic drugs is illustrated below:

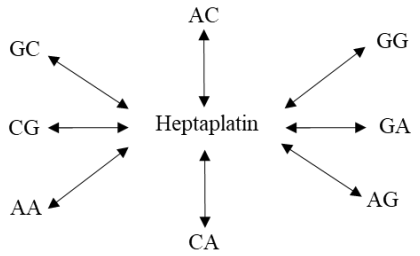
$$GG > GA > GC > AG$$

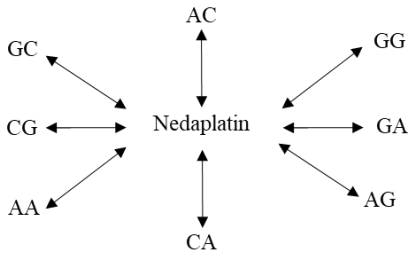
In order to better understand the interactions with platinum drugs and DNA, the five most common platinum drugs were introduced to various dinucleotides containing G and A sites (See **Table 1**).

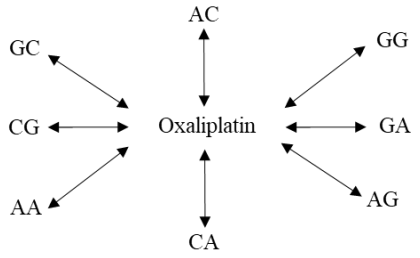
Table 1. **Drug to DNA Interaction Diagrams**
This table shows different DNA to drug interactions that were examined throughout this thesis. In the middle are the five platinum drugs and the arrows indicate the various DNA the drug was interacted with.











CHAPTER III

METHODOLOGIES

A. Sample Preparation

A1. DNA Reconstitution

The DNA samples were provided by BioSYNTHESIS in a nanomole (nmol) concentration. Since the DNA samples were less than 8 bases, the reconstitution procedure was simplified. The sample vials containing DNA were centrifuged for about 30 seconds to ensure that all contents were settled at the base of the tube. Ultrapure water was then added so that the concentration of DNA within the vial was 10^{-3}M. The DNA was then vortexed for a few seconds to mix the solution then was centrifuged again for another 30 seconds. The DNA was then stored at 4°C.

A2. Drug Preparation

The drug stock bottles were received from LKT Laboratories Inc. The drugs come in powdered form and need to be dissolved in solution. Since carboplatin and cisplatin exist in a powdered form and are highly toxic if inhaled, a nitrogen inflated glove bag is used to weigh out each drug. Calculations are completed to know the amount (grams) of drug needed to be suspended in ultrapure water to make the desired concentration of drug. For SERS samples—the drugs need to be 10^{-5}M and for HPLC—the drugs need to be 10^{-2}M. Once the solutions are made – they can be used for approximately 2 months. The finished samples were stored at 4°C until ready to use.

A3. Silver Nanoparticle Synthesis

Aqua Regia:

The purpose of using Aqua Regia is to clean the glassware that will hold the silver nanoparticles. Aqua Regia, also known as nitrohydrochloric acid, was prepared by combining nitric acid (HNO_3) and hydrochloric acid (HCl) at a ratio of 1:3 (300mL HCl and 100mL HNO_3). It is important to introduce the HNO_3 slowly to HCl because heat is generated from the reaction. The solution was kept under a fume hood uncovered for approximately 3 hours. Once complete, the aqua Regia was transferred to all glassware used in the experiment and set aside to ensure all contaminants were removed.

Figure 9. The above picture illustrates the synthesis of Silver Nanoparticles.

Silver Nanoparticles:

A combined mixture of 400mL ultrapure water and 90mg of pre-dissolved $AgNO_3$ was stirred and gradually heated. Once the mixture came to a boil, 100mg of dissolved sodium citrate was added. The mixture then underwent a color change or clear to yellow to brown. Once the solution displayed a brown tint, the solution was boiled for 30 more minutes. Once the solution was through boiling, water was added to bring the final volume

to 400mL. The silver nanoparticle solution was set aside to cool. Here, another color change occurred, the solution became a greenish/gray color (**Figure 9**).

Aqua Regia Disposal:

Once the silver nanoparticles were synthesized and ready for storage, the Aqua Regia was separated into several large beakers full of water and sodium bicarbonate for dilutions and cooled. Once the Aqua Regia had been carefully and thoroughly diluted, it was washed down the sink with running water and sodium bicarbonate. The amber bottle was then rinsed with ultrapure water 4 times and the silver nanoparticles were added.

B. SERS and Raman

B1. Adenine + Cisplatin and Carboplatin

SERS Samples:

Old:

180µl adenine (10^{-5}) + 36µl Drug (10^{-4}) + 500µl Nanoparticles + 200µl $MgSO_4$
100µl adenine (10^{-5}) + 100µl Drug (10^{-4}) + 500µl Nanoparticles + 200µl $MgSO_4$
100µl adenine (10^{-5}) + 100µl Drug (10^{-5}) + 500µl Nanoparticles + 200µl $MgSO_4$

New:

150µl adenine (10^{-4}) + 150µl Drug (10^{-4}) + 500µl Nanoparticles + 200µl $MgSO_4$
250µl adenine (10^{-4}) + 50µl Drug (10^{-4}) + 500µl Nanoparticles + 200µl $MgSO_4$

Figure 10 shows the silver nanoparticles aggregated at the bottom of the sample vials. With the new protocol, the pH of adenine must be adjusted once the solution is made and prior to incubation. The pH of adenine in a water solution is roughly 8.7. 1M HCl is used to lower the pH so that it mimics the normal pH within the body. Once the pH of adenine is brought down to roughly 7, a more realistic interaction can be monitored when the drug is introduced.

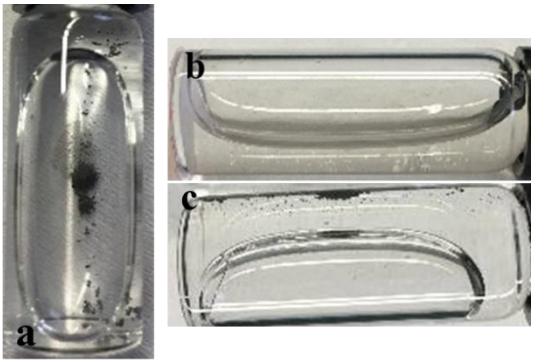


Figure 10. The figures illustrate the aggregated nanoparticles within the samples. (a) Nanoparticles clustered in the center of a sample vial. (b) Newley aggregated nanoparticles. (c) Clustered nanoparticles within a sample vial.

Incubation of Samples

Since the interaction of drug to DNA is observed outside of the body, it is crucial to allow the interaction to occur in the same environment as it would in the body. Each sample prepared is incubated at 37°C (body temperature) to allow the interaction to fully take place as it under the relative same conditions as if in the body. For the old protocol each sample was incubated for approximately 5 hours each. For the new protocol, the samples were incubated for 24 hours and 48 hours.

B2. DNA + Platinum Drugs

The platinum drug samples prepared were carried out using the same methods as the adenine + cisplatin and carboplatin. For the DNA samples, the DNA was reconstituted following the DNA Reconstitution method previously mentioned.

100µl DNA (10^{-5}) + 100µl Drug (10^{-4}) + 500µl Nanoparticles + 200µl $MgSO_4$

C. HPLC

C1. DNA + Platinum Drugs

Since the sample volume being injected into the HPLC is low (10μL), the sample sizes are small. The sample synthesis for DNA and cisplatin, nedaplatin, and oxaliplatin is given below:

6μl DNA (10^{-3}) + 6μl Drug (10^{-2})

The sample synthesis for DNA and carboplatin is given below:

6μl DNA (10^{-3}) + 12μl Drug (10^{-2})

Incubation of Samples

Since the interaction of drug to DNA is observed outside of the body, it is crucial to allow the interaction to occur in the same environment as it would in the body. Each sample prepared is incubated for 24hrs. at 37°C (body temperature) to allow the interaction to fully take place as it under the relative same conditions as if in the body.

D. Data Collection and Experimental Conditions

D1. SERS and Raman

Once the nanoparticles and salt were added to the sample after incubation, time was allotted so the sample could aggregate, approximately 30-60 minutes. Once aggregation occurred, cyclohexane was used to calibrate the instrument prior to sample observation. **Figure 11** shows the DXR Raman instrument used in this research.

Experimental Parameters:

Laser Wavelength: 532 nm
Laser Power: 2.0 mW
Aperture: 25 µm pinhole
Grating: 900 lines/min
Estimated Resolution: 2.7-4.2 cm^{-1}
Estimated Spot Size: 2.1 µm
Allowed Range: 3563 to 4 cm^{-1}
Min Range Limit: 50 cm^{-1}
Max Range Limit: 3404 cm^{-1}
Objective: MPlan 10x/0.25 BD
Number of Scans: 100

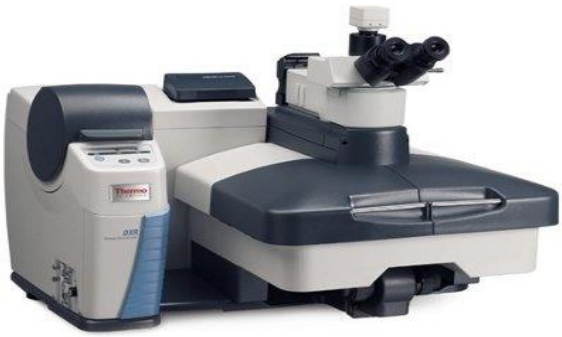


Figure 11. The figure above illustrates the Raman Instrument used throughout the experiment. DXR Raman Microscope with a laser power of 532nm.

Normal Raman:

A small sample of DNA or drug in its powdered form was placed on a glass microscope slide and observed under a 532nm laser radiation.

D2. HPLC

Mobile Phase: The triethylammonium acetate (TEAA) solution was purchased from Glenn Research. The solution is HPLC grade, has a pH 7 and is at a concentration of 2M. The solution was then degassed to ensure no bubbles were present then connected to the HPLC instrument. A stock solution of ultra-pure water was obtained and degassed

before being introduced to the HPLC instrument. The solution parameters were set at a 80:20 (TEAA:H_2O). The gradient solvents were ran through the system for about 30min, to allow full introduction to the HPLC.

Experimental conditions:

Stationary Phase: The HPLC was equipped with a C_{18} column.
Detector: UV-Vis detector
Light Source: D_2 and Tungsten lamps
Sample: The sample injection volume was set to 10μL.

CHAPTER IV

SERS OF ADENINE WITH CISPLATIN AND CARBOPLATIN

This chapter covers the first project of this thesis, SERS of the DNA base adenine with cisplatin and carboplatin. As previously mentioned in Chapter II, the difference between SERS and normal Raman is that with SERS, metal colloids are used to increase the Raman signal. As stated in the sample preparation chapter, silver colloids were used in the SERS samples. In **Figure 12** below, the difference in spectral intensity and resolution can be observed for adenine through SERS and normal Raman. **Figure 13** along with **Table 2** show the interaction between carboplatin and adenine and **Figure 14** along with **Table 3** show the interaction between cisplatin and adenine. It is well known that platinum drugs prefer to bind with guanine over adenine. As mentioned in the introduction chapter of this thesis, platinum prefers to bind to purine bases (adenine and guanine) at the N7 terminal. However, guanine platination is more thermodynamically and kinetically more favorable.[30] The expected results from this project were to see little interaction between both carboplatin and cisplatin with adenine. The data analysis for SERS is primarily based on peak assignments where each peak is related to a certain vibrational mode that can give more information on the interaction(s) occurring through observation of peak shifts. **Tables 2** and **3** show the assignments through comparing the peak shifts from adenine to the peak shifts of the complex of adenine with either cisplatin or carboplatin. From this, the delta

shift was calculated. With the Raman instruments used, the resolution is 5cm^{-1} so if the delta shift is within 5cm^{-1}, it can be concluded that no significant modifications were made to adenine by the platinum drug.

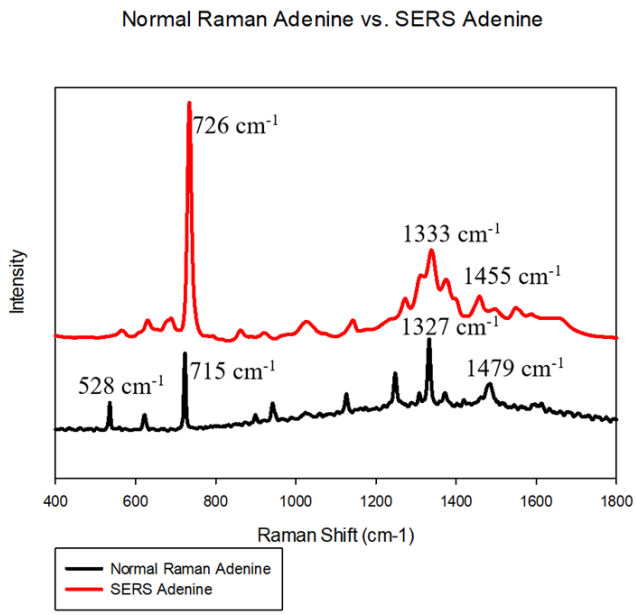


Figure 12. Illustrates the comparison between a normal Raman spectrum of adenine (black) and a SERS spectrum of adenine (red)

As previously mentioned, when metal colloids are added to a sample the Raman signal is increased by as much as 10^8. **Figure 12** shows how the intensity and clarity of a spectrum can be improved upon colloid addition. As can be observed from the spectra of Normal Raman v. SERS of adenine, the signal produced from the Normal Raman contained more noise than SERS. It is known for Normal Raman signals to display weaker properties because only one in a million photons will scatter inelastically.[8] Normal Raman displays smaller bandwidths, so identifying individual peaks can be done more accurately. There is

a slight shift in peak placement when comparing the SERS spectrum to the Normal Raman.

This occurrence could be a result from the noise present in the Normal Raman spectrum.

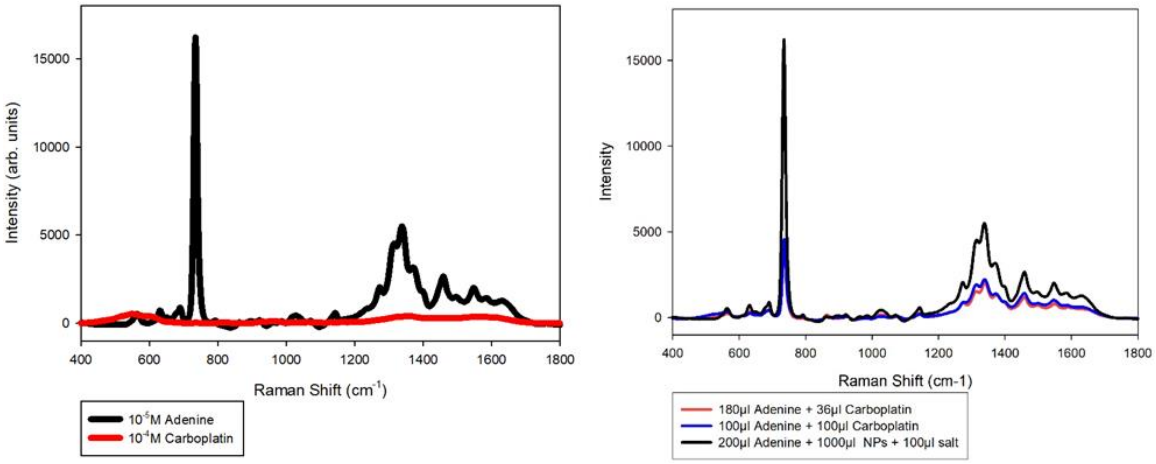


Figure 13. Shows the comparison between SERS spectra of adenine, carboplatin, and adenine + carboplatin complexes. (left) Illustrates the comparison in SERS spectra on adenine and carboplatin. (right) Illustrates the comparison of adenine and adenine + carboplatin complexes at a 1:1 and a 1:5 molar ratio.

Table 2. Gives the Δ shift of key peaks within the SERS spectra to compare the vibrational changes between the adenine spectrum and the adenine + carboplatin spectrum. The resolution of our Raman system is $5cm^{-1}$ so any Δ shift within $5cm^{-1}$ is considered insignificant. The Δ shift values indicate that there were deformations (δ) made to adenine by carboplatin in the 5 (R5) and 6 (R6) membered ring.[22]

Raman Shift (cm^{-1})			
Adenine	Adenine + Carboplatin 1:5	Δ shift	Assignments
1269.12	1272.3	-3.18	δ R5, R6
1315.6	1316.16	-0.56	δ R5, R6
1345	1343.84	1.16	δ R5, R6
1455.6	1457.76	-2.16	δ R5, R6

Having carboplatin present does not display significant changes in spectral shifts. By looking at the Δ shift values, each value is below 5cm^{-1} indicating no significant changes to adenine by carboplatin. The interaction between carboplatin and adenine is weak.

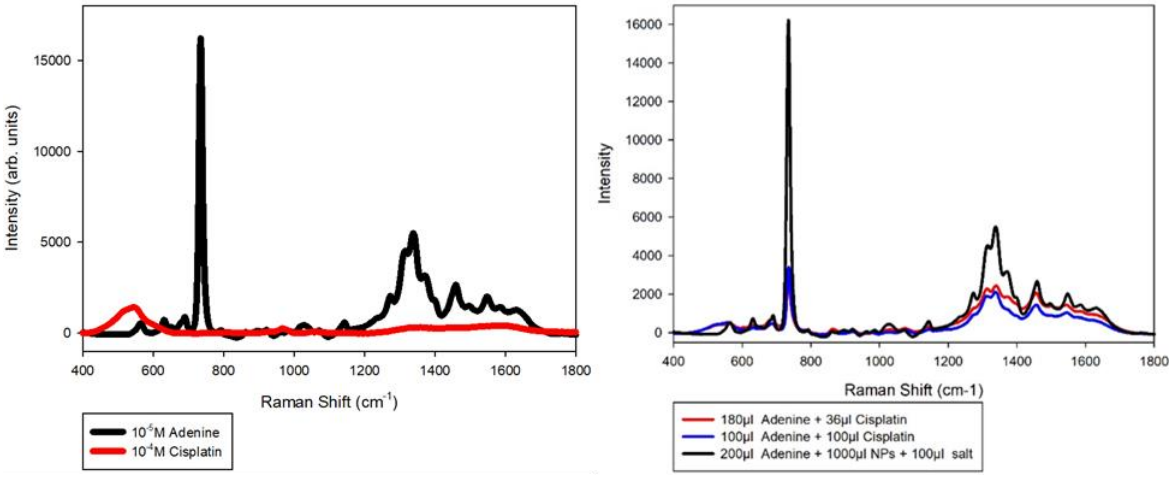


Figure 14. Shows the comparison between SERS spectra of adenine, cisplatin, and adenine + cisplatin complexes. (left) Illustrates the comparison in SERS spectra on adenine and cisplatin. (right) Illustrates the comparison of adenine and adenine + cisplatin complexes at a 1:1 and a 1:5 molar ratio.

Table 3. **Gives the Δ shift of key peaks within the SERS spectra to compare the vibrational changes between the adenine spectrum and the adenine + cisplatin spectrum.** The resolution is 5cm^{-1} so any Δ shift within 5cm^{-1} is considered insignificant. The Δ shift values indicate that there were deformations (δ) made to adenine by cisplatin in the 5 (R5) and 6 (R6) membered rings.[22]

Raman Shift (cm^{-1})			
Adenine	Adenine + Cisplatin 1:5	Δ shift	Assignments
1269.12	1273.28	-4.16	δ R5, R6
1315.6	1313.28	2.32	δ R5, R6
1345	1340	5	δ R5, R6
1455.6	1456.64	-1.04	δ R5, R6

Looking at the spectra above some peaks disappeared or shifted when comparing adenine to the adenine and cisplatin complexes. The adenine-cisplatin peaks near 1200-1700cm^{-1} were not as defined compared to the adenine spectrum. There is relatively more interaction between cisplatin and adenine when compared to carboplatin because of the altered peaks. The Δ shifts seen in **Table 3** are closer to the resolution of our Raman system that is about 5cm^{-1}, therefore, these shifts are still relatively insignificant.

In a study conducted by Otto et al[21] in 1986, a similar adenine SERS spectrum was obtained using a low powered helium-neon laser and peak identifications were assigned. In another study produced by Koglin et al[20] in 1984, adenine was suspended in silver colloids (without drug) at a concentration of 1.5×10^{-6}M at a pH of 4.5. A similar basic adenine spectrum was established, and peak assignments were given. Östblom et al,[22] observed the structural desorption of DNA bases adsorbed on gold colloids in a recent publication.

Conclusion

The data presented in this chapter agrees with previous research and publications[30] that platinum does not prefer to interact with adenine. Primarily looking at the data tables for Δ shifts, the values needed to be much greater than the resolution 5cm^{-1} to identify any significant modifications made to adenine by the platinum drugs, but all values were within 5cm^{-1}. **Tables 2** and **3** indicate that there were slight deformations to adenine, but no major modifications occurred.

CHAPTER V

OBSERVING MODIFICATIONS MADE TO GG, GA, AND AG DNA BY CISPLATIN AND CARBOPLATIN BY EMPLOYING SERS

From the data obtained in the previous chapter and since knowing that platinum prefers to bind with guanine over adenine, in this chapter, more complex samples were examined. In this research project, GG, GA, and AG DNA was introduced to both cisplatin and carboplatin and observed using SERS. The decision to use the three previously listed DNA is due to the trend observed by Sheardy and coworkers and has been discussed in Chapter II that cisplatin prefers to interact with GG over GA, GC, and AG.[14] One of the major questions driving this research is finding if the above trend is true for all platinum drugs or just cisplatin and if there are any new trends that can be discovered. Answers to these questions are closely monitored in this chapter and the next employing HPLC technique. **Figures 15**, **16**, **18**, and **20** demonstrate spectra of cisplatin and DNA complexes and **Figures 17**, **19**, and **21** show spectra of carboplatin and DNA complexes.

Like in the previous chapter, silver colloids were synthesized and used in each SERS sample in increase the Raman intensity and use of a very low concentration of samples. In order to preform our experiments similar to that of Chapter VI, the following

SERS samples were obtained at a 1:1 DNA to drug volume ratio (refer to Chapter III- B2 for sample preparation).

Similar to Chapter IV, the data analysis for SERS samples is based on the peak shifts (Δ shift). **Tables 4-9** show the peak wavenumber of the original peak in the DNA spectrum and the peak in the DNA and drug complex. The peaks are compared by the Δ shift then assigned to a vibrational mode. The peak wavenumbers are from the spectra produced in the project and the assignments are from several reference tables.[31,32,33] This analysis is so the interaction between the drug and DNA can be better understood by observing the vibrational modifications the drug makes to the DNA. The resolution for Raman is $5cm^{-1}$, so if the Δ shift is within $5cm^{-1}$, the change is not considered significant.

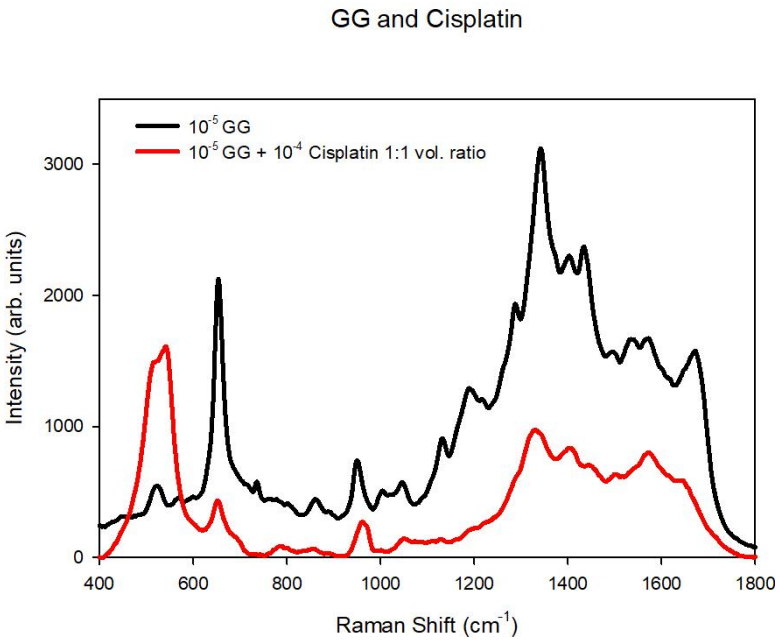


Figure 15. Illustrates the SERS spectra of GG and GG+cisplatin complex.

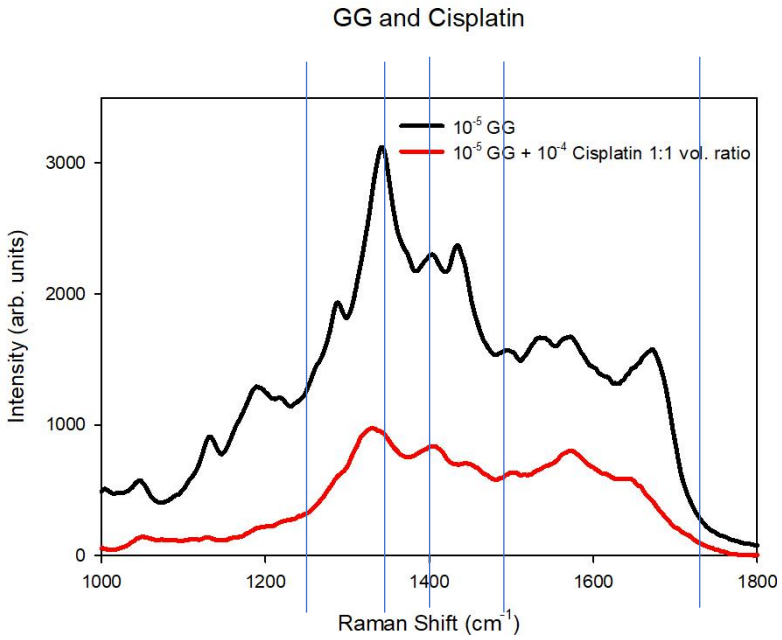


Figure 16. Is the GG and GG+cisplatin spectra above, but focused on the region of 1000cm-1 to 1800cm-1 to clearly show the modifications being made to GG.

Table 4. **Raman shift values of SERS spectra of GG and GG+cisplatin within 5cm^{-1} resolutions and peak assignments to vibrational modes.**[31,32,33] The resolution is 5cm^{-1} so any Δ shift within 5cm^{-1} is considered insignificant.

Raman Shift (cm^{-1})			Assignment(s)
GG	GG + cisplatin 1:1 vol. ratio	Δ shift	
653.4	655.36	-1.96	in-ring breathing mode of G
953.28	959.44	-6.16	
1342.76	1330.44	12.32	skeletal ring-vibrations mode
1401.28	1409.4	-8.12	
1569.84	1574.04	-4.2	A, G
1674	1649.08	24.92	

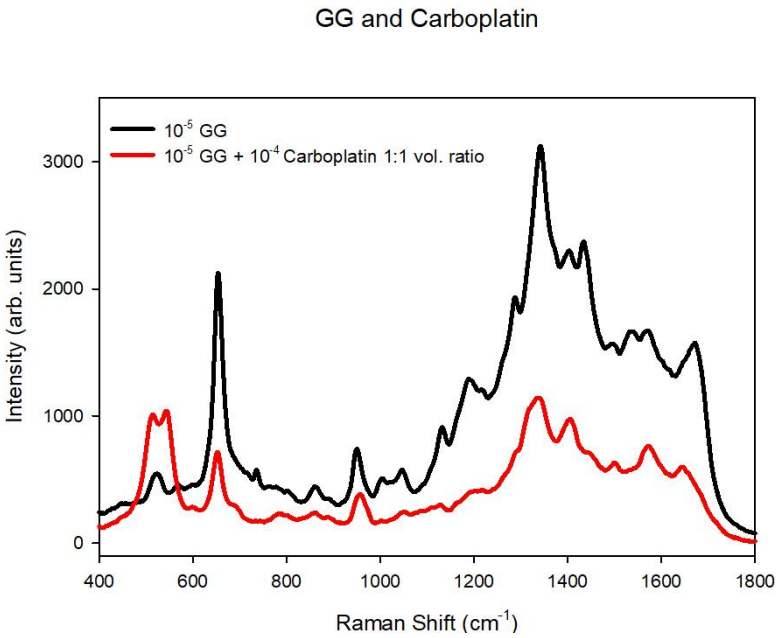


Figure 17. Illustrates the SERS spectra of GG and GG+carboplatin complex.

Table 5. Raman shift values of SERS spectra of GG and GG+carboplatin within 5cm^{-1} resolutions and peak assignments to vibrational modes.[31,32,33] The resolution is 5cm^{-1} so any Δ shift within 5cm^{-1} is considered insignificant.

Raman Shift (cm^{-1})			Assignment(s)
GG	GG + carboplatin 1:1 vol. ratio	Δ Shift	
653.4	653.68	-0.28	in-ring breathing mode of G
953.28	958.88	-5.6	
1342.76	1342.48	0.28	skeletal ring-vibrations mode
1401.28	1407.44	-6.16	
1569.84	1577.96	-8.12	A, G
1674	1647.4	26.6	

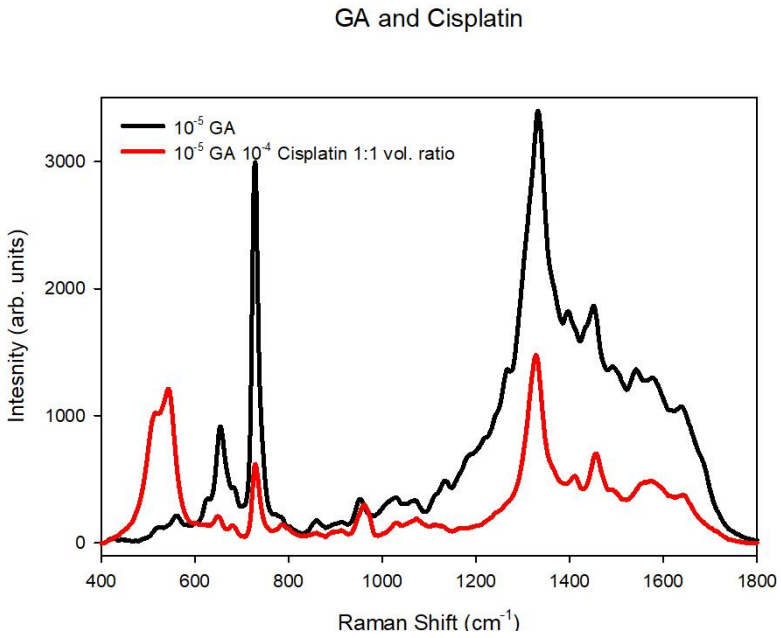


Figure 18. Illustrates the SERS spectra of GA and GA+cisplatin complex.

Table 6. Raman shift values of SERS spectra of GA and GA+cisplatin within 5cm^{-1} resolutions and peak assignments to vibrational modes.[31,32,33] The resolution is 5cm^{-1} so any Δ shift within 5cm^{-1} is considered insignificant.

Raman Shift (cm^{-1})			Assignment(s)
GA	GA + cisplatin 1:1 vol. ratio	Δ Shift	
655.92	655.92	0	in-ring breathing mode of G
729.84	732.36	-2.52	A, ring breathing
954.4	963.36	-8.96	NH2 group vibration on A
1028.32	1030.56	-2.24	
1333.52	1329.04	4.48	skeletal ring-vibrations mode
1400.72	1416.4	-15.68	
1452.24	1456.72	-4.48	vibration mode of deoxyribose and A
1582.44	1573.48	8.96	A, G
1640.68	1647.4	-6.72	

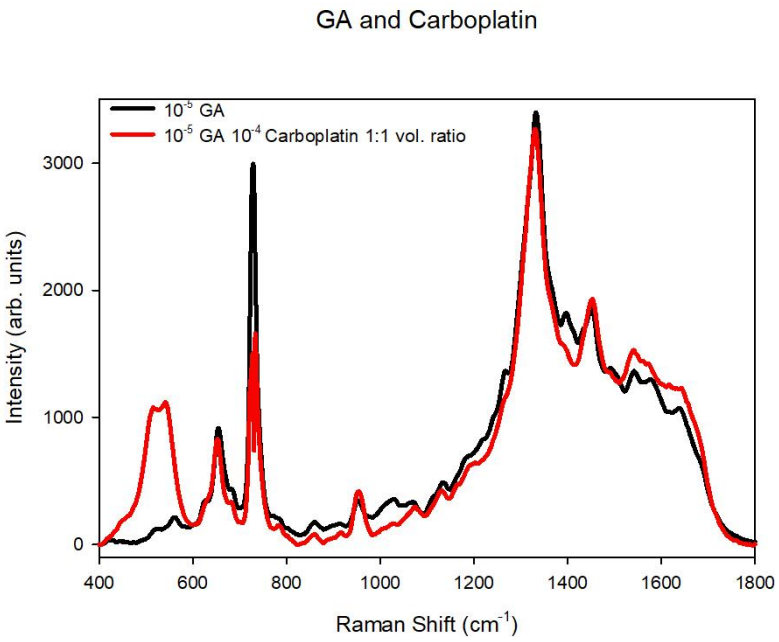


Figure 19. Illustrates the SERS spectra of GA and GA+carboplatin complex.

Table 7. Raman shift values of SERS spectra of GA and GA+carboplatin within 5cm^{-1} resolutions and peak assignments to vibrational modes.[31,32,33] The resolution is 5cm^{-1} so any Δ shift within 5cm^{-1} is considered insignificant.

Raman Shift (cm^{-1})			Assignment(s)
GA	GA + carboplatin 1:1 vol. ratio	Δ Shift	
655.92	652.84	3.08	in-ring breathing mode of G
729.84	728.16	1.68	A, ring breathing
954.4	956.64	-2.24	NH2 group vibration on A
1028.32	1075.64	-47.32	
1136.12	1133.88	2.24	in-bending C8-H, str C4-H9, rock NH2 of G
1333.52	1328.48	5.04	skeletal ring-vibrations mode
1452.24	1450	2.24	vibration mode of deoxyribose and A
1544.36	1539.88	4.48	
1640.68	1646.84	-6.16	

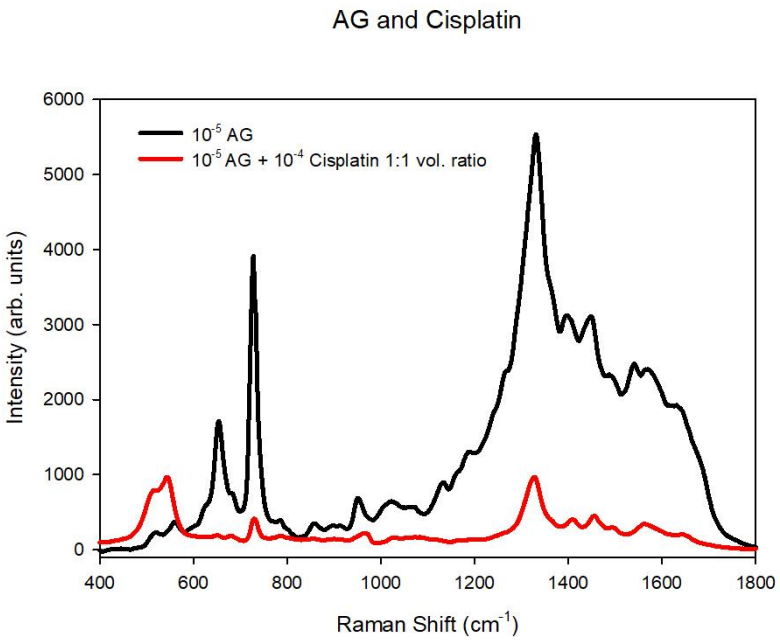


Figure 20. Illustrates the SERS spectra of AG and AG+cisplatin complex.

Table 8. Raman shift values of SERS spectra of AG and AG+cisplatin within 5cm^{-1} resolutions and peak assignments to vibrational modes.[31,32,33] The resolution is 5cm^{-1} so any Δ shift within 5cm^{-1} is considered insignificant.

Raman Shift (cm^{-1})			Assignment(s)
AG	AG + cisplatin 1:1 vol. ratio	Δ Shift	
657.04			in-ring breathing mode of G
726.2	732.36	-6.16	A, ring breathing
951.6	970.08	-18.48	NH2 group vibration on A
1327.92	1329.04	-1.12	skeletal ring-vibrations mode
1396.8	1414.16	-17.36	
1455.32	1454.48	0.84	vibration mode of deoxyribose and A
1574.6	1560.04	14.56	A, G
1638.16	1651.88	-13.72	

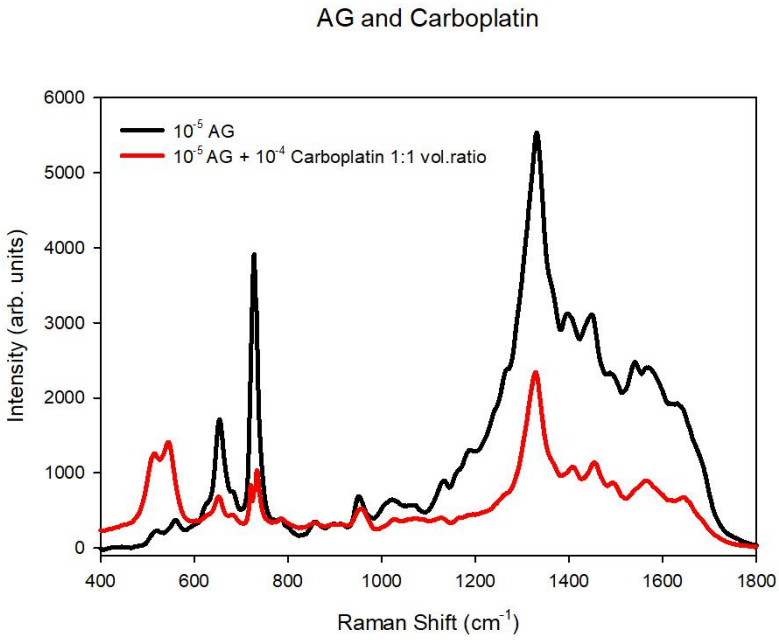


Figure 21. Illustrates the SERS spectra of AG and AG+carboplatin complex.

Table 9. **Raman shift values of SERS spectra of AG and AG+carboplatin within 5cm^{-1} resolutions and peak assignments to vibrational modes.**[31,32,33] The resolution is 5cm^{-1} so any Δ shift within 5cm^{-1} is considered insignificant.

Raman Shift (cm^{-1})			Assignment(s)
AG	AG + carboplatin 1:1 vol. ratio	Δ Shift	
657.04	654.52	2.52	in-ring breathing mode of G
726.2	734.04	-7.84	A, ring breathing
951.6	961.96	-10.36	NH2 group vibration on A
1327.92	1333.24	-5.32	skeletal ring-vibrations mode
1396.8	1410.24	-13.44	
1455.32	1457.84	-2.52	vibration mode of deoxyribose and A
1574.6	1566.48	8.12	A, G
1638.16	1648.8	-10.64	

Conclusion

Judging by the visual changes to each spectrum and the Δ shifts from each complex, cisplatin made more modifications to GG and AG than it did GA and carboplatin modified AG and GG more than GA. The trend gathered for cisplatin modifications to DNA is found

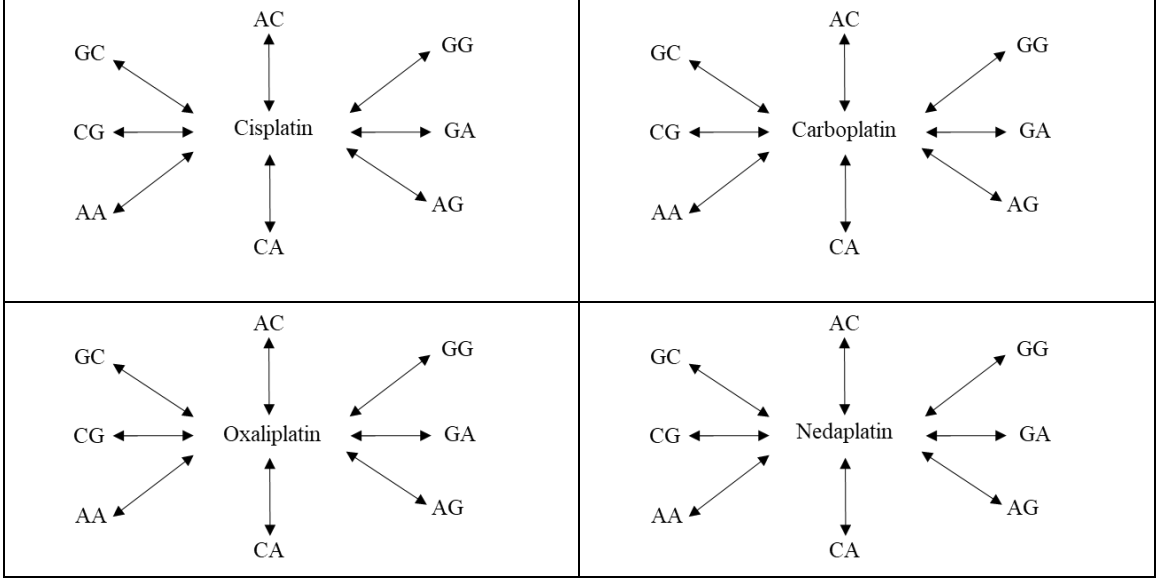
to be GG>AG>GA and for carboplatin AG>GG>GA. Overall, cisplatin interacted most with each of the DNA than carboplatin did. The cisplatin trend produced from this project did not fully agree with the previous known trend of its binding preference to be GG>GA>GC>AG seen in the work of Sheardy and coworkers.[14] In the next chapter, the same DNA observed in this chapter is also examined with two other platinum drugs, nedaplatin and oxaliplatin using High-Performance Liquid Chromatography (HPLC).

CHAPTER VI

USING RP-HPLC TO OBSERVE INTERACTIONS BETWEEN AA, AC, AG, CA, CG, GA, GC, GG, AND CARBOPLATIN, CISPLATIN, HEPTAPLATIN, NEDAPLATIN, AND OXALIPLATIN

In this chapter, the interaction between platinum drugs and DNA was further examined using a new method to the lab group – high-performance liquid chromatography (HPLC). More specifically, this project focused on the interactions between four popular platinum chemotherapeutic drugs – carboplatin, cisplatin, nedaplatin, and oxaliplatin with eight DNA dinucleotides – AA, AC, AG, CA, CG, GA, GC, and GG. All of these interactions are shown in **Table 10** below:

Table 10. **Shows the interactions monitored using HPLC.** The Platinum drug is located in the center and is surrounded by the various DNA they were introduced to.



Since incorporating HPLC into this research project was new, rigorous research went into developing a protocol. One publication that aided in creating the HPLC protocol was by Harrington et al.[28] This publication focused on 1,2 intrastrand guanine-guanine DNA and its interaction with cisplatin. The researchers employed HPLC combined with ICP-MS in order to find the limit of detection (LOD) and the limit of quantification for the GG + cisplatin complex. The idea of using triethylammonium acetate (TEAA), the column, and detector was also collected from the research produced by Harrington et al. One other publication that majorly contributed to the development of this HPLC protocol was research conducted by Kozelka et al.[29] Research done by Kozelka et al studied GG and AG DNA adducts and their interaction with cisplatin. The adducts were detected using HPLC.

In order to gain skills and knowledge regarding HPLC in this research project, experimental parameters were set up to gradually optimize the protocol. At first, sample ratios were based off volume ratios. Samples were made at a 1:1 DNA to drug ratio to test the protocol and efficiency of the HPLC instrument. Samples were also incubated for 24 hours at 37°C before introduction to the instrument. **Figures 30-53** are HPLC chromatograms with a 1:1 DNA to drug volume ratio, but **Figures 22-29** were obtained at a 1:2 DNA to drug volume ratio because when carboplatin interacted with the DNA at the original 1:1 ratio no spectral changes were observed. As previously mentioned, TEAA was chosen as part of the mobile phase for HPLC experiments. 2M TEAA was combined with ultrapure H_2O at a gradient of 80:20 TEAA to H_2O. At the start of this method, 2M TEAA was synthesized in the lab, but as demand for it grew with the continuation of experiments, the TEAA was ordered as a premade stock solution. Since HPLC, is a highly sensitive and

selective analytical technique, the slightest variations within the mobile phase (pH, molarity, etc.) may cause the chromatogram to be slightly shifted causing data to be somewhat different from the previous set. Which leads to the following **Figures 22-53**; there are two chromatographs side-by-side, Run 1 and Run 2. The data plots for Run 1 were obtained from the same premade stock solution of 2M TEAA and the data plots for Run 2 were obtained from the same premade stock solution of 2M TEAA, but a different stock solution from Run 1. Both data sets are present to compare how the slightest variations within the mobile phase can alter the results to a small degree.

The main analysis of the data was quantitative. The peak areas (area under the curve) were obtained and used to carry out several calculations to determine how much of the DNA was reacted with the drug. The following is a sample calculation where $Area_{uc}$ is area under the curve, A_{run1} and A_{run2} are the areas under the curve in Run 1 and Run 2, $A_{DNApeakcmplx}$ is the area under the curve of the DNA peak in the complex, and $A_{DNApeak}$ is the area under the curve of the DNA peak by itself:

$$\boldsymbol{A}reauc = \frac{\boldsymbol{A}run1 + \boldsymbol{A}run2}{2} = \boldsymbol{A}avg$$

$$\left(\frac{\boldsymbol{A}DNApeakcmplx}{\boldsymbol{A}DNApeak}\right) x100 = \%\, of\ DNA\ remaining$$

The calculations show what percentage of the DNA was left unbound to the drug and the percentage of the drug that was not reactive with the DNA. The data set that is most promising is the percentage of DNA left unbound. The data showing the percentage of the drug left unbound is still significant data, but due to peak overlapping gave high, inaccurate percentages. **Tables 11-18** show the peak areas for each drug and DNA, peak areas for the

specified DNA and drug peak within each complex, and the percentage of the drug or DNA that was left unbound within the complex.

Carboplatin + DNA

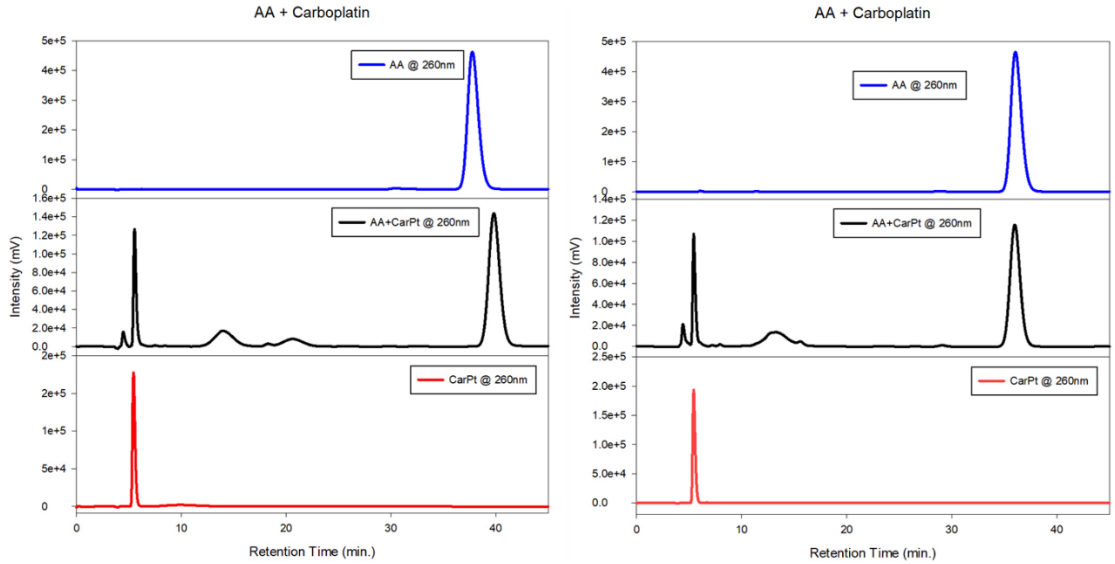


Figure 22. Illustrates two runs of AA DNA, carboplatin, and AA+carboplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

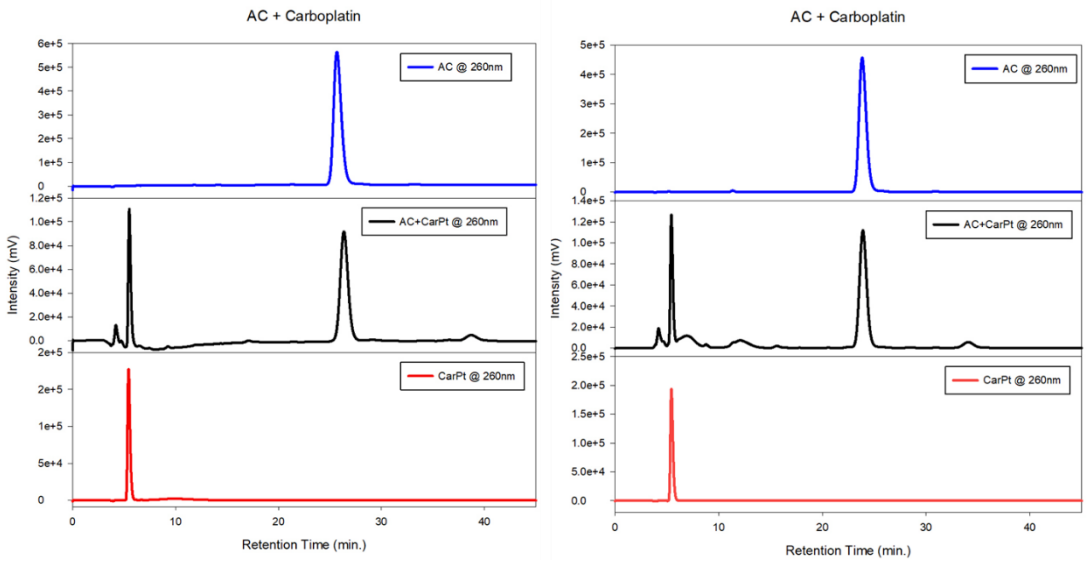


Figure 23.Illustrates two runs of AC DNA, carboplatin, and AC+carboplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

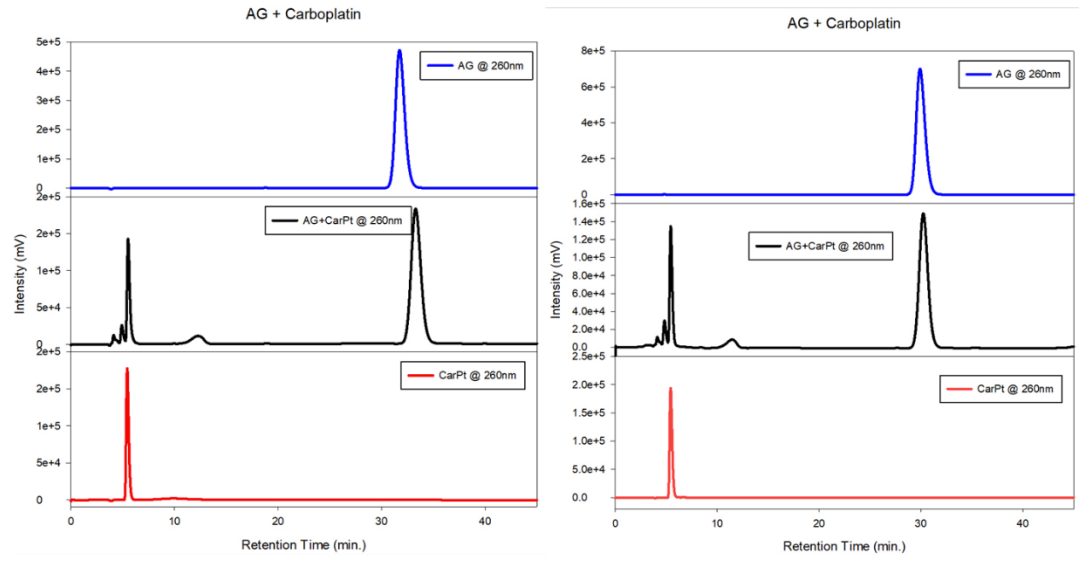


Figure 24. Illustrates two runs of AG DNA, carboplatin, and AG+carboplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

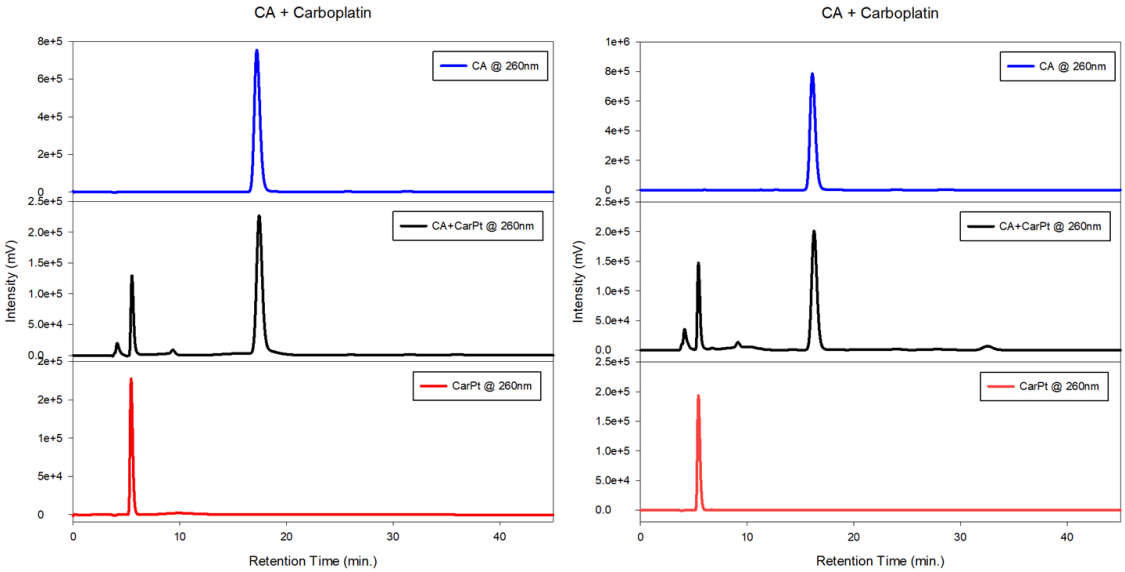


Figure 25.Illustrates two runs of CA DNA, carboplatin, and CA+carboplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

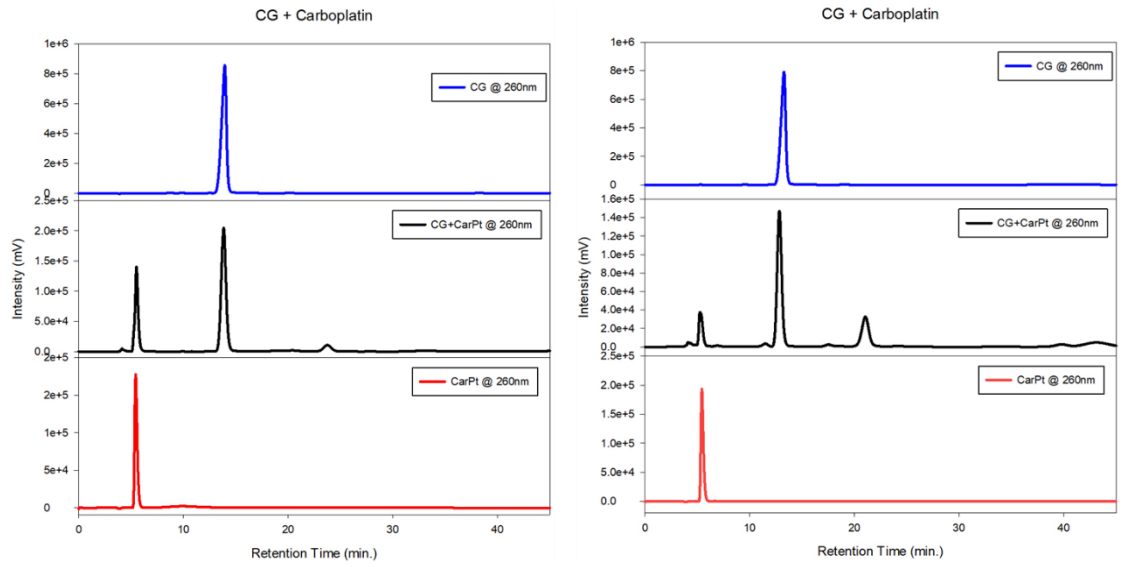


Figure 26.Illustrates two runs of CG DNA, carboplatin, and CG+carboplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

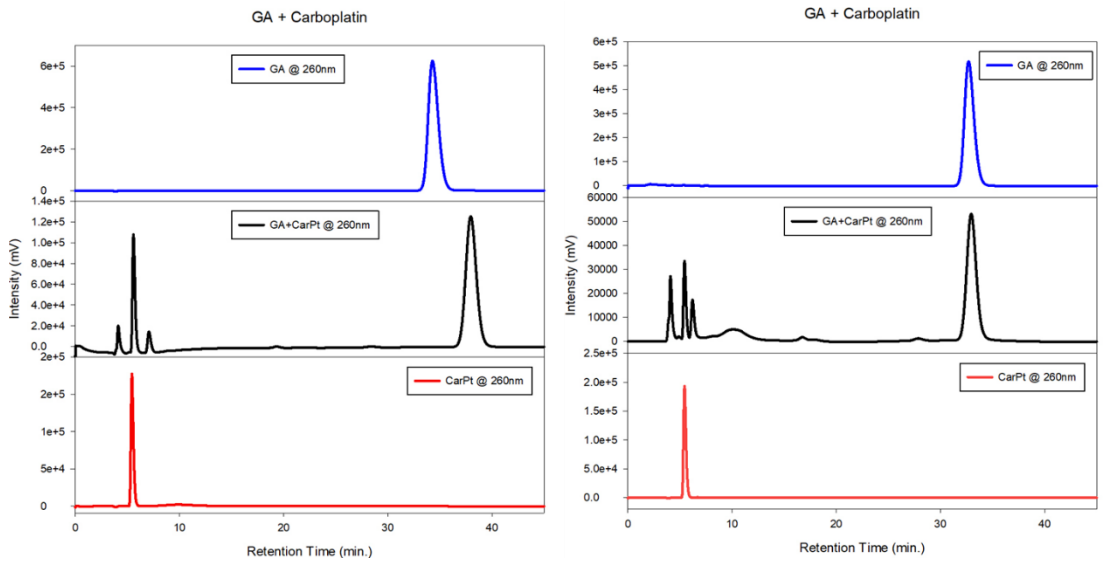


Figure 27. Illustrates two runs of GA DNA, carboplatin, and GA+carboplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

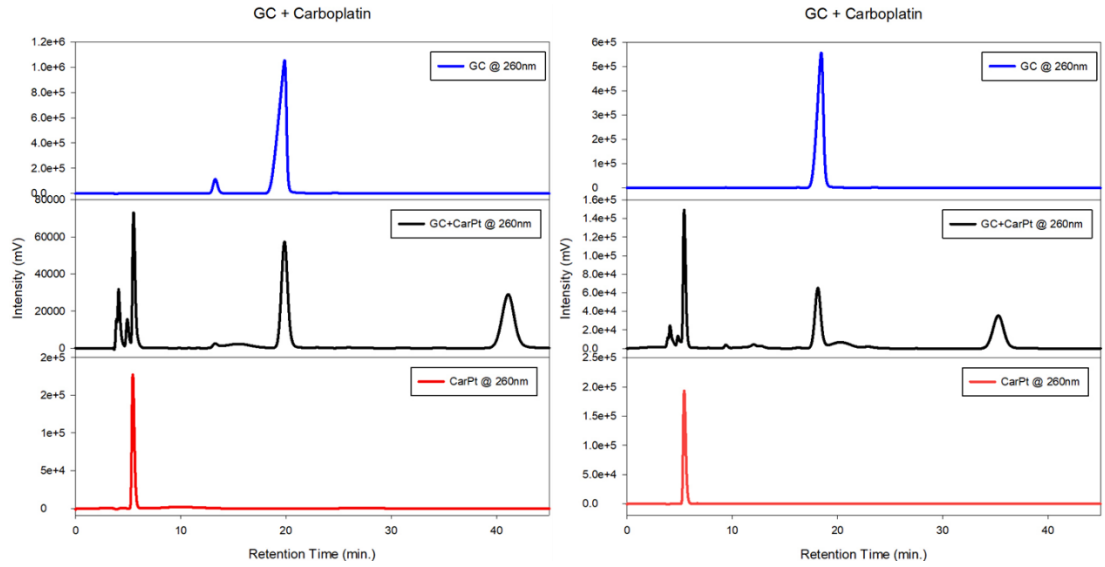


Figure 28. Illustrates two runs of GC DNA, carboplatin, and GC+carboplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

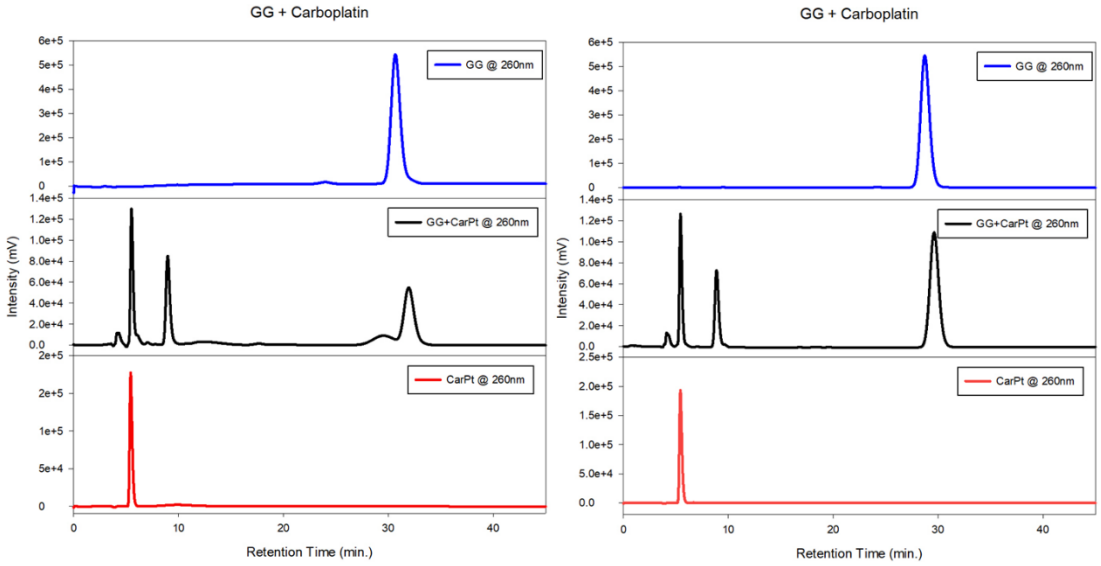


Figure 29. Illustrates two runs of GG DNA, carboplatin, and GG+carboplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

Table 11. Illustrates the calculations performed for HPLC data analysis. The calculations show the average peak areas for the individual DNA, the DNA peak(s) within the complex, and the % of DNA left unbound within the complex. The drugs are abbreviated carboplatin (Car), cisplatin (Cis), nedaplatin (Ned), and oxaliplatin (Ox). Each complex is shown with the DNA and the abbreviated drug (AA_Car signifies AA DNA in a complex with carboplatin).

	Area Under the Curve Calculations for DNA and Complexes:								
DNA Peak Areas (arb. units)		AA	AC	AG	CA	CG	GA	GC	GG
	run 1	33779407	30449737	29887987	29234149	28917872	43180184	61701948	33934769
	run 2	33423958	23411526	43647026	28705600	25882319	34052588	34052588	33200992
	Avg	33601683	26930632	36767507	28969875	27400096	38616386	47877268	33567881
DNA Peak Areas Within Complex (arb. units)		AA_Car	AC_Car	AG_Car	CA_Car	CG_Car	GA_Car	GC_Car	GG_Car
	run 1	10890001	5695112	12317524	9483054	6800263	9206347	2652316	3956469
	run 2	8034905	5617994	9186663	7203658	4570866	3533024	2714170	6689496
	Avg	9462453	5656553	10752094	8343356	5685565	6369686	2683243	5322983
% of DNA Left Unbound in Complex		AA_Car	AC_Car	AG_Car	CA_Car	CG_Car	GA_Car	GC_Car	GG_Car
		28.2	21.0	29.2	28.8	20.8	16.5	5.6	15.9

Table 12. Illustrates the calculations performed for HPLC data analysis. The calculations show the average peak areas for the individual drug, the drug peak(s) within the complex, and the % of drug left unbound within the complex. The drugs are abbreviated carboplatin (Car), cisplatin (Cis), nedaplatin (Ned), and oxaliplatin (Ox). Each complex is shown with the DNA and the abbreviated drug (AA_Car signifies AA DNA in a complex with carboplatin).

	Area Under the Curve Calculations for Drug and Complexes								
Drug Peak Areas (arb. units)		Car	Cis	Neda	Ox				
	run 1	3437663	523116	1798236	18549237				
	run 2	3446879	453120	2307234	17845406				
	Avg	3442271	488118	2052735	18197322				
Drug Peak Areas Within Complex (arb. units)		AA_Car	AC_Car	AG_Car	CA_Car	CG_Car	GA_Car	GC_Car	GG_Car
	run 1	2723742	2116109	3143154	3393554	3049779	2116171	1710350	2956927
	run 2	2210119	2320614	2561634	2667151	986338	605244	2744341	2342858
	Avg	2466931	2218362	2852394	3030353	2018059	1360708	2227346	2649893
% of Drug Left Unbound in Complex		AA_Car	AC_Car	AG_Car	CA_Car	CG_Car	GA_Car	GC_Car	GG_Car
		71.7	64.4	82.9	88.0	58.6	39.5	64.7	76.9

The carboplatin samples were made as a 1:2 DNA:drug volume ratio because no spectral changes were observed at a 1:1 ratio. So, volumetrically speaking, there was twice as much carboplatin present than there was DNA. This explains why in most complexes there was a significant percentage of unbound carboplatin. After many experiments using several volume ratios, the data shown in the chromatograph remained fairly consistent in that the DNA appeared almost unaffected by carboplatin. The binding specificity for platinum with DNA is known to be GG > GA > GC > AG so expected results would assume there would be a smaller percentage of unbound GG and a higher percentage of unbound AG. In the data set there are some variations to this trend as the GG+carboplatin complex yields ~16% of unbound DNA to carboplatin, GA+carboplatin complex yields ~16.5% of unbound DNA, GC+carboplatin complex yields ~6% of unbound DNA, and the AG+carboplatin complex yields ~29% of unbound DNA.

Cisplatin + DNA

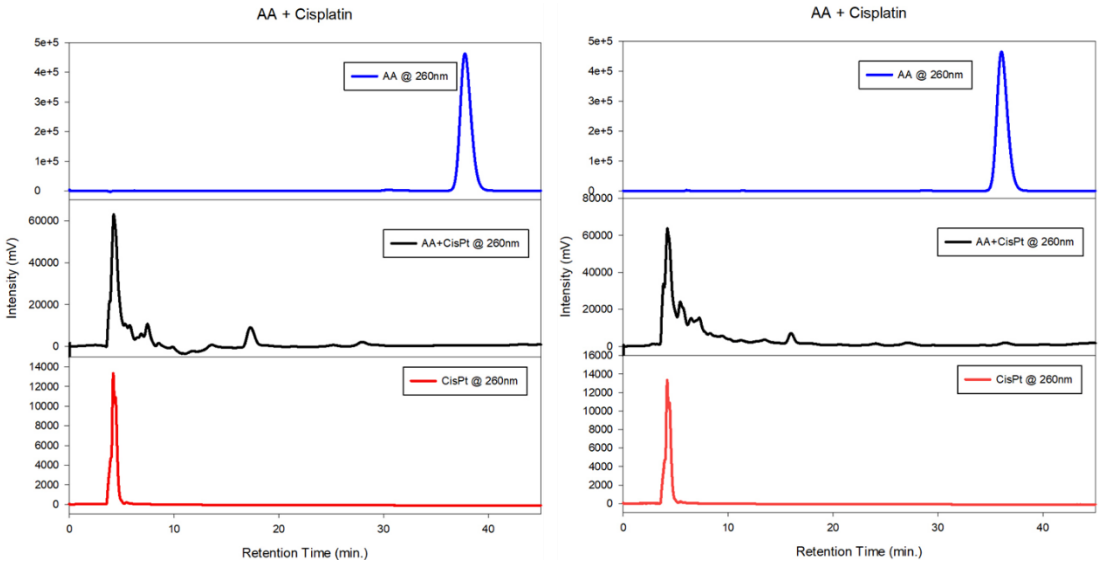


Figure 30. Illustrates two runs of AA DNA, cisplatin, and AA+cisplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

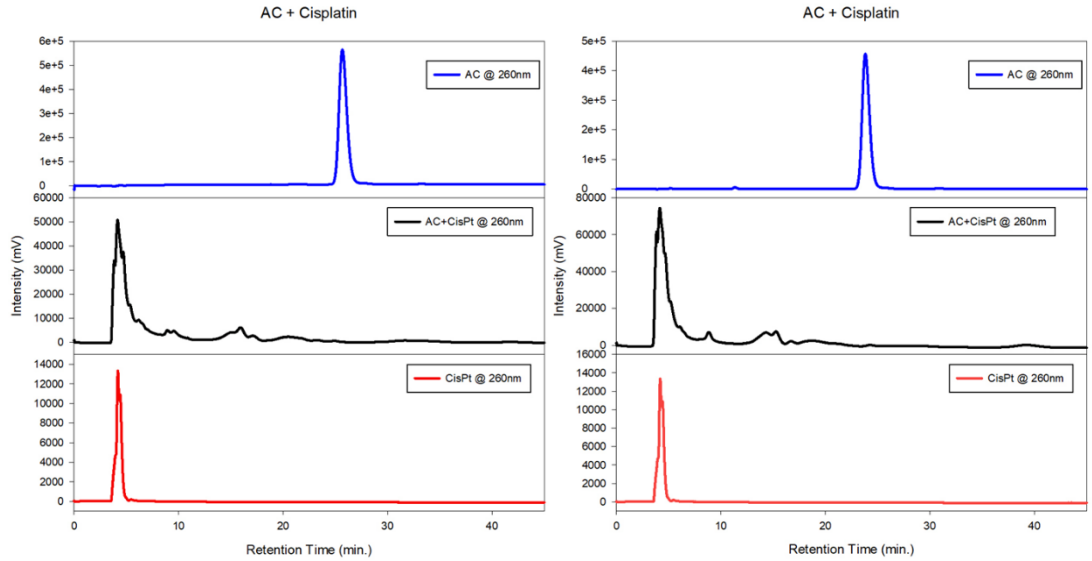


Figure 31. Illustrates two runs of AC DNA, cisplatin, and AC+cisplatin complex. All scans were obtained using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

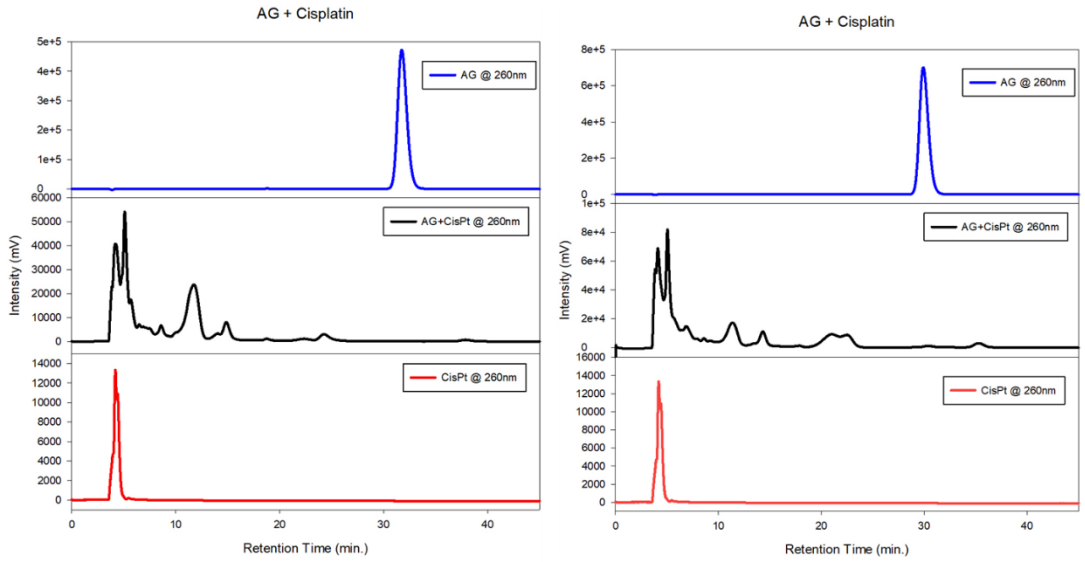


Figure 32. Illustrates two runs of AG DNA, cisplatin, and AG+cisplatin complex. All scans were obtained using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

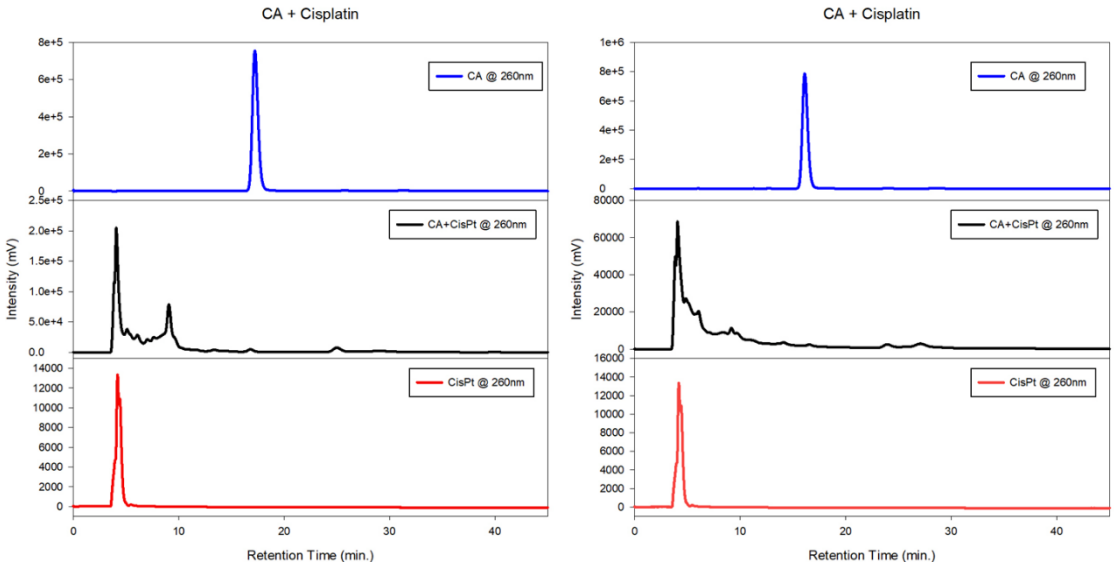


Figure 33. Illustrates two runs of CA DNA, cisplatin, and CA+cisplatin complex. All scans were taken using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

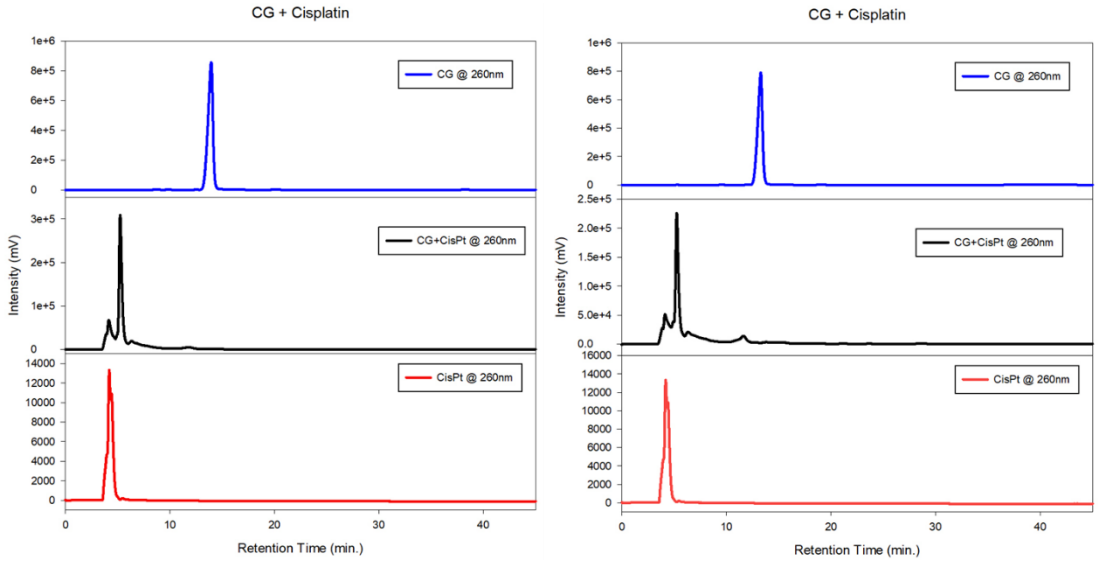


Figure 34. Illustrates two runs of CG DNA, cisplatin, and CG+cisplatin complex. All scans were obtained using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

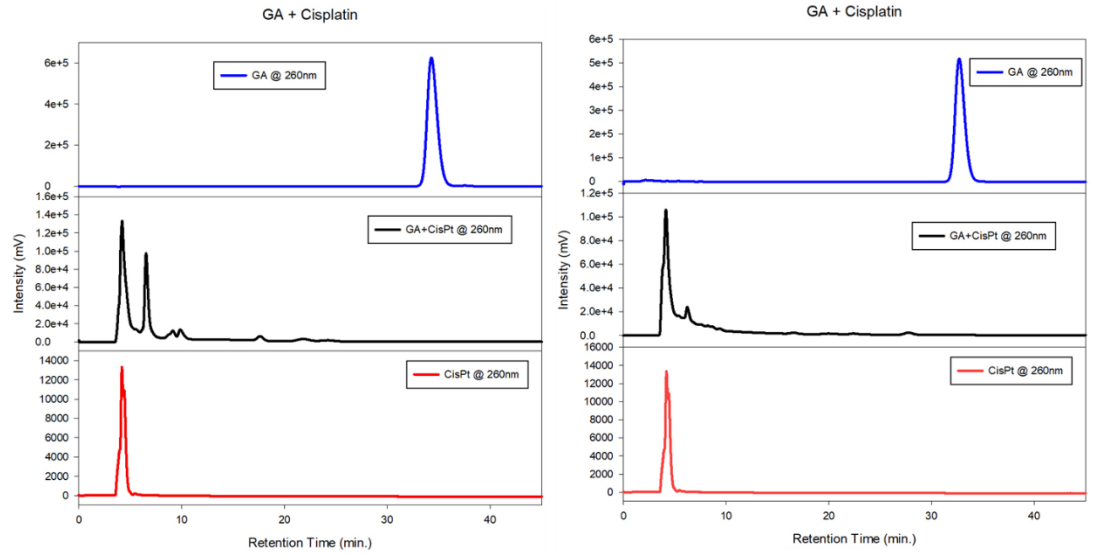


Figure 35. Illustrates two runs of GA DNA, cisplatin, and GA+cisplatin complex. All scans were taken using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

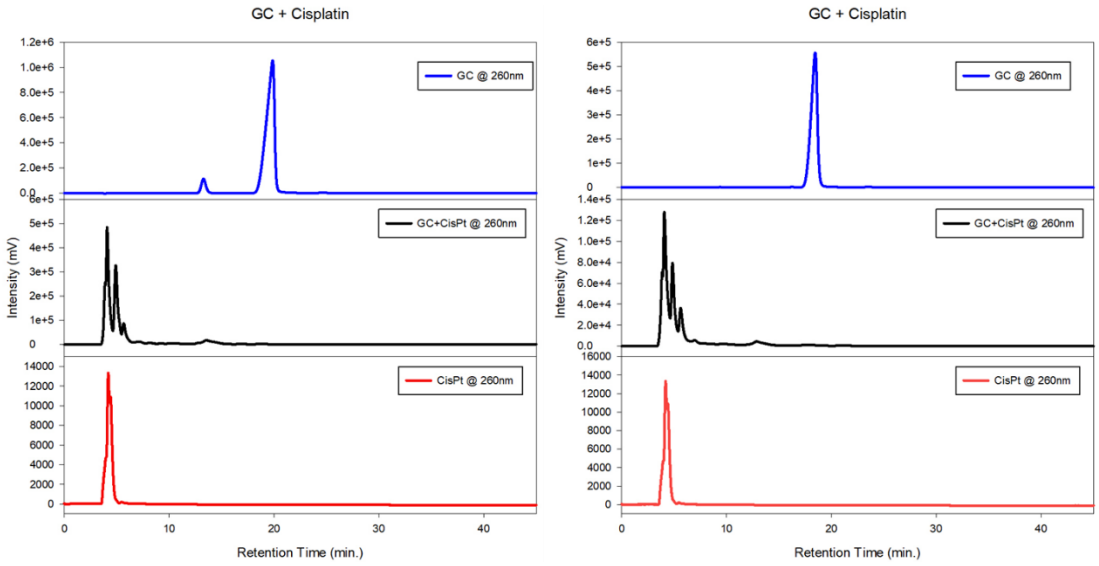


Figure 36. Illustrates two runs of GC DNA, cisplatin, and GC+cisplatin complex. All scans were obtained using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

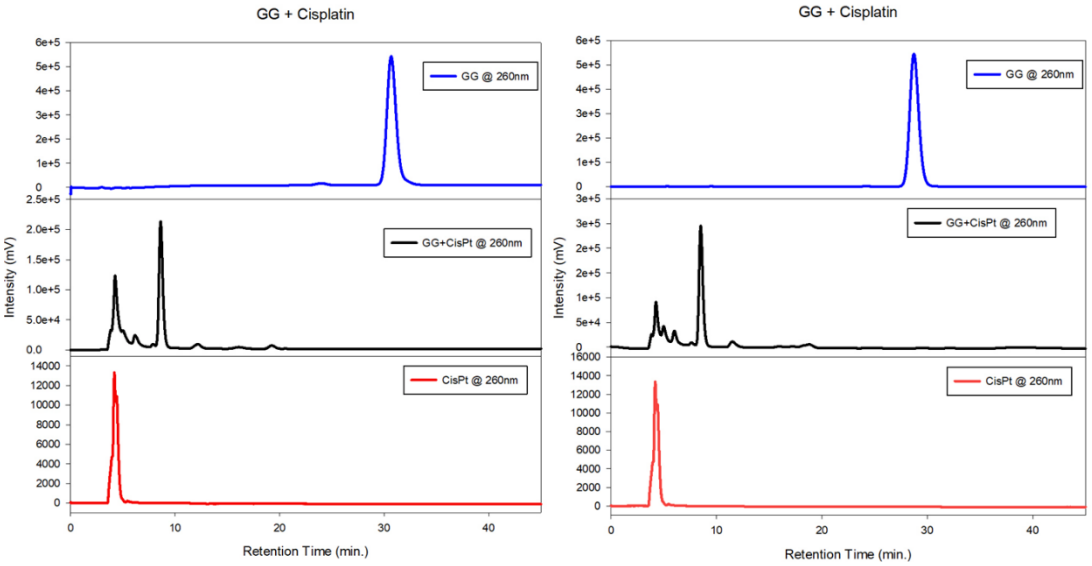


Figure 37. Illustrates two runs of GG DNA, cisplatin, and GG+cisplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

Table 13. Illustrates the calculations performed for HPLC data analysis. The calculations show the average peak areas for the individual DNA, the DNA peak(s) within the complex, and the % of DNA left unbound within the complex. The drugs are abbreviated carboplatin (Car), cisplatin (Cis), nedaplatin (Ned), and oxaliplatin (Ox). Each complex is shown with the DNA and the abbreviated drug (AA_Cis signifies AA DNA in a complex with cisplatin).									
	Area Under the Curve Calculations for DNA and Complexes:								
DNA Peak Areas (arb. units)		AA	AC	AG	CA	CG	GA	GC	GG
	run 1	33779407	30449737	29887987	29234149	28917872	43180184	61701948	33934769
	run 2	33423958	23411526	43647026	28705600	25882319	34052588	34052588	33200992
	Avg	33601683	26930632	36767507	28969875	27400096	38616386	47877268	33567881
DNA Peak Areas Within Complex (arb. units)		AA_Cis	AC_Cis	AG_Cis	CA_Cis	CG_Cis	GA_Cis	GC_Cis	GG_Cis
	run 1	6279	15442	0	152355	32047	8675	1744964	12949
	run 2	79155	191720	118603	131557	175275	0	31265	38611
	Avg	42717	103581	59301.5	141956	103661	4337.5	888114.5	25780
% of DNA Left Unbound in Complex		AA_Cis	AC_Cis	AG_Cis	CA_Cis	CG_Cis	GA_Cis	GC_Cis	GG_Cis
		0.13	0.38	0.16	0.49	0.38	0.01	1.9	0.08

Table 14. Illustrates the calculations performed for HPLC data analysis. The calculations show the average peak areas for the individual drug, the drug peak(s) within the complex, and the % of drug left unbound within the complex. The drugs are abbreviated carboplatin (Car), cisplatin (Cis), nedaplatin (Ned), and oxaliplatin (Ox). Each complex is shown with the DNA and the abbreviated drug (AA_Cis signifies AA DNA in a complex with cisplatin).									
	Area Under the Curve Calculations for Drug and Complexes:								
Drug Peak Areas (arb. units)		Car	Cis	Neda	Ox				
	run 1	3437663	523116	1798236	18549237				
	run 2	3446879	453120	2307234	17845406				
	Avg	3442271	488118	2052735	18197322				
Drug Peak Areas Within Complex (arb. units)		AA_Cis	AC_Cis	AG_Cis	CA_Cis	CG_Cis	GA_Cis	GC_Cis	GG_Cis
	run 1	3013294	1641094	1828421	6440170	2652356	5616345	12532442	3791943
	run 2	2807809	4523968	2253964	2744112	1778455	5160091	2954991	2523140
	Avg	2910552	3082531	2041193	4592141	2215406	5388218	7743717	3157542
% of Drug Left Unbound in Complex		AA_Cis	AC_Cis	AG_Cis	CA_Cis	CG_Cis	GA_Cis	GC_Cis	GG_Cis
		596.3	631.5	418.2	940.8	453.9	1103.9	1586.4	646.9

With experiments involving cisplatin, the volume ratio was 1:1 DNA:drug while obtaining satisfactory results. The percentages of drug unbound within the complex is exceptionally high, this could be a result of peak overlap within the chromatograph. When cisplatin was individually introduced through the HPLC, one defined peak appeared, but when it was added to DNA, the peak area and intensity were increased resulting in a broader peak. Closely observing the early peaks within each plot, there is evidence that two or three more peaks could be overlapping the cisplatin peak causing the percentage of unbound drug to be significantly high. In regard to the known trend, there was also some variations in the results, but less than carboplatin complexes. GG+cisplatin complex yielded ~ 0.08% unbound DNA, GA+cisplatin yielded ~ 0.01% unbound DNA, GC+cisplatin yielded ~ 1.9% unbound DNA, and AG+cisplatin yielded ~ 0.16% unbound DNA.

Nedaplatin + DNA

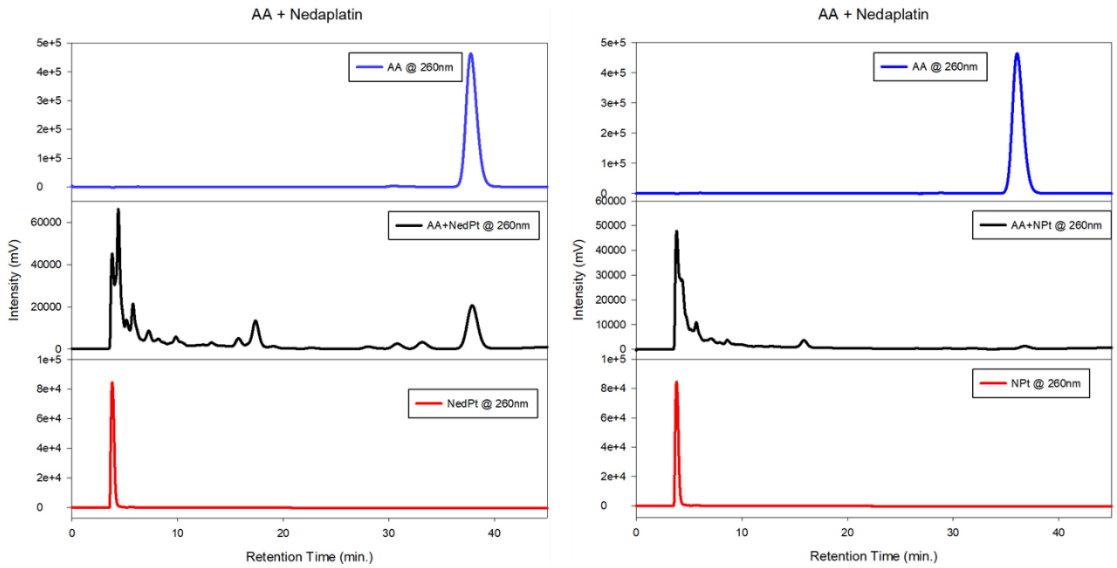


Figure 38. Illustrates two runs of AA DNA, nedaplatin, and AA+nedaplatin complex. All scans were taken using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

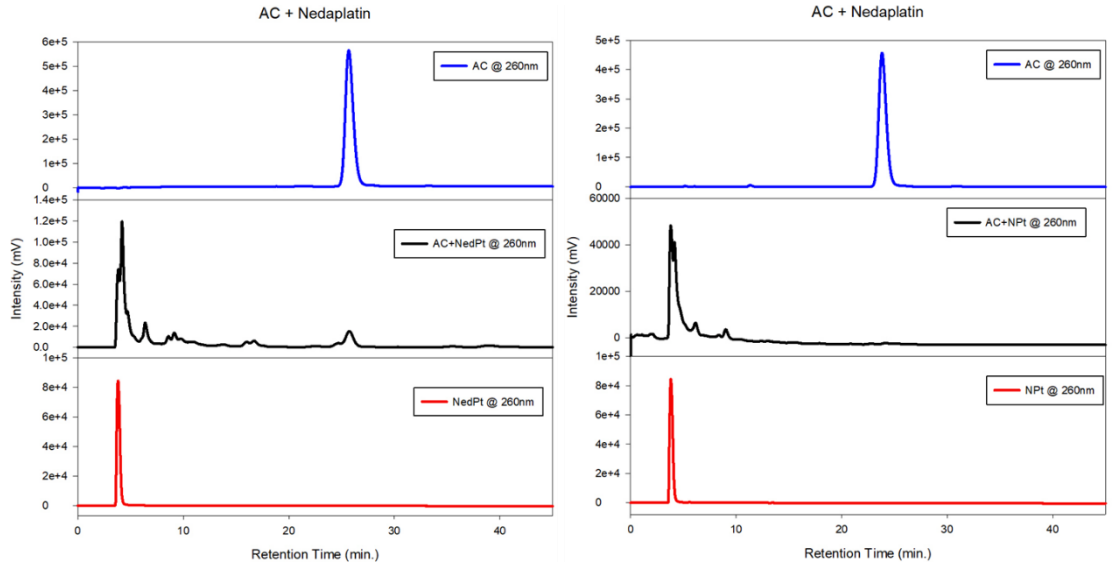


Figure 39. Illustrates two runs of AC DNA, nedaplatin, and AC+nedaplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

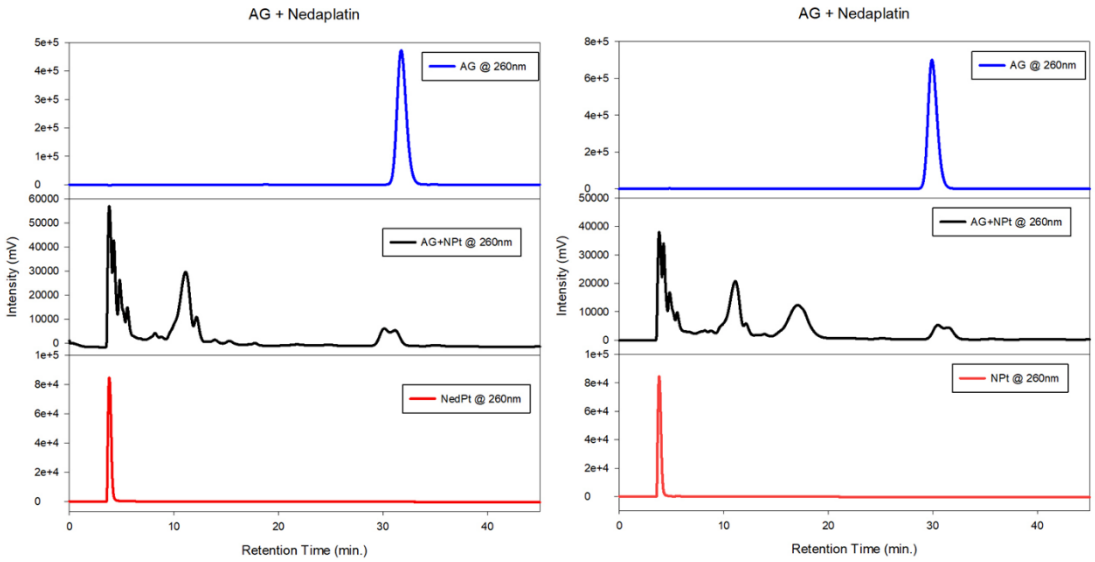


Figure 40. Illustrates two runs of AG DNA, nedaplatin, and AG+nedaplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

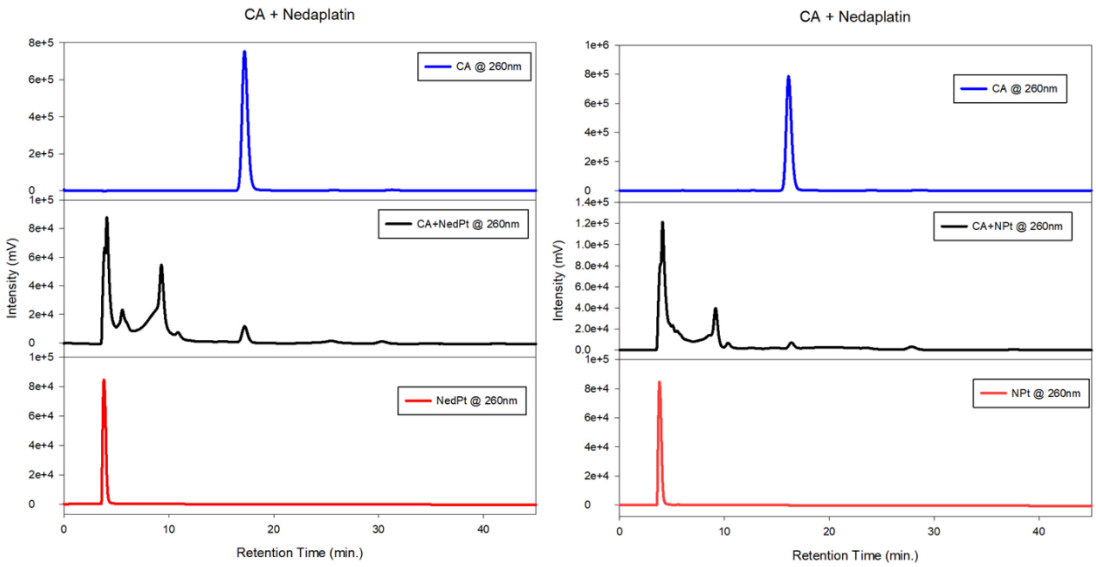


Figure 41. Illustrates two runs of CA DNA, nedaplatin, and CA+nedaplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

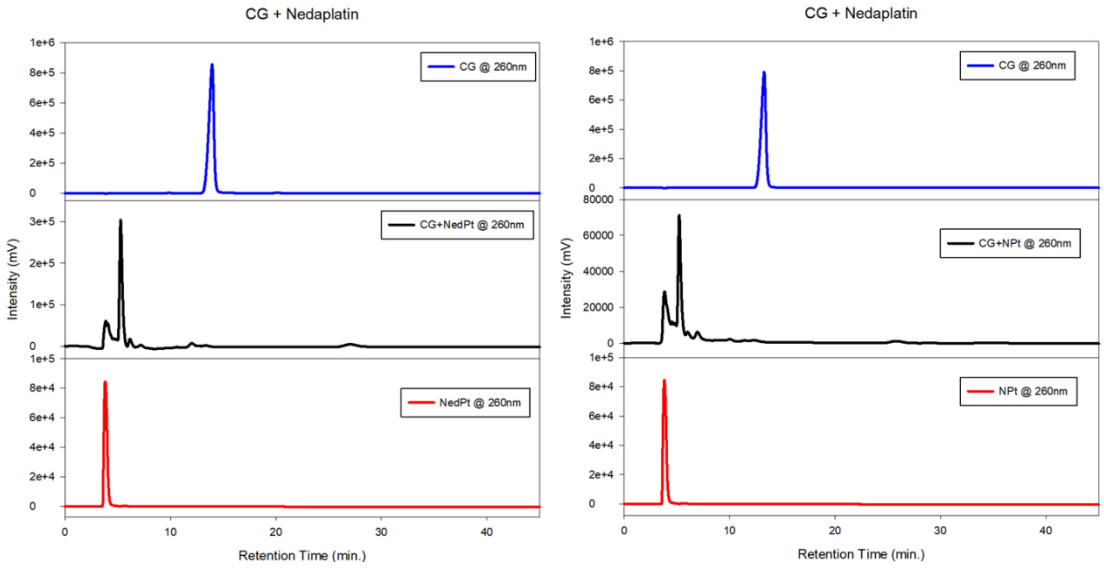


Figure 42. Illustrates two runs of CG DNA, nedaplatin, and CG+nedaplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

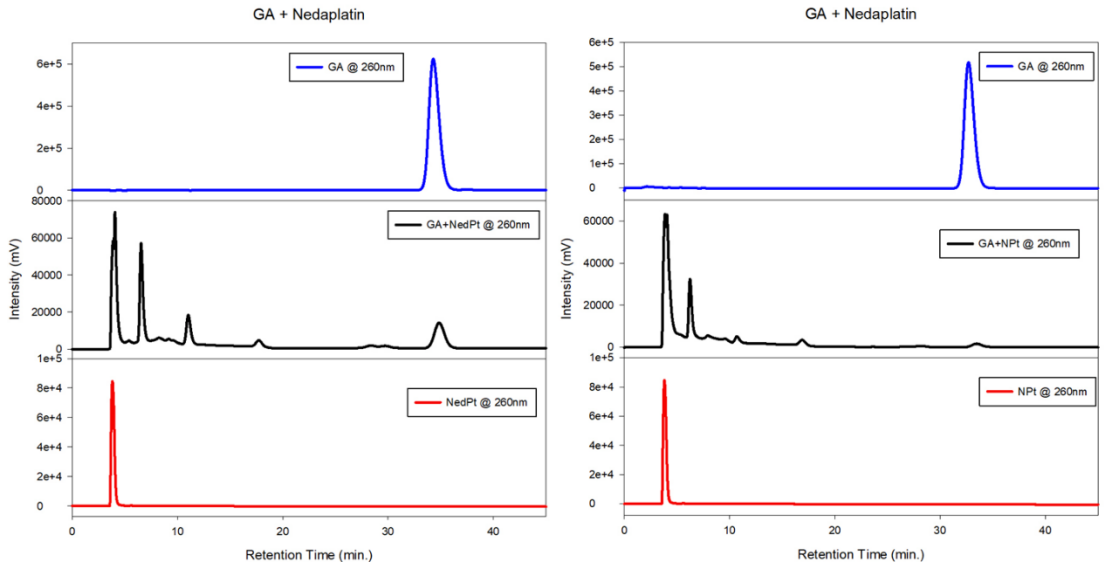


Figure 43. Illustrates two runs of GA DNA, nedaplatin, and GA+nedaplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

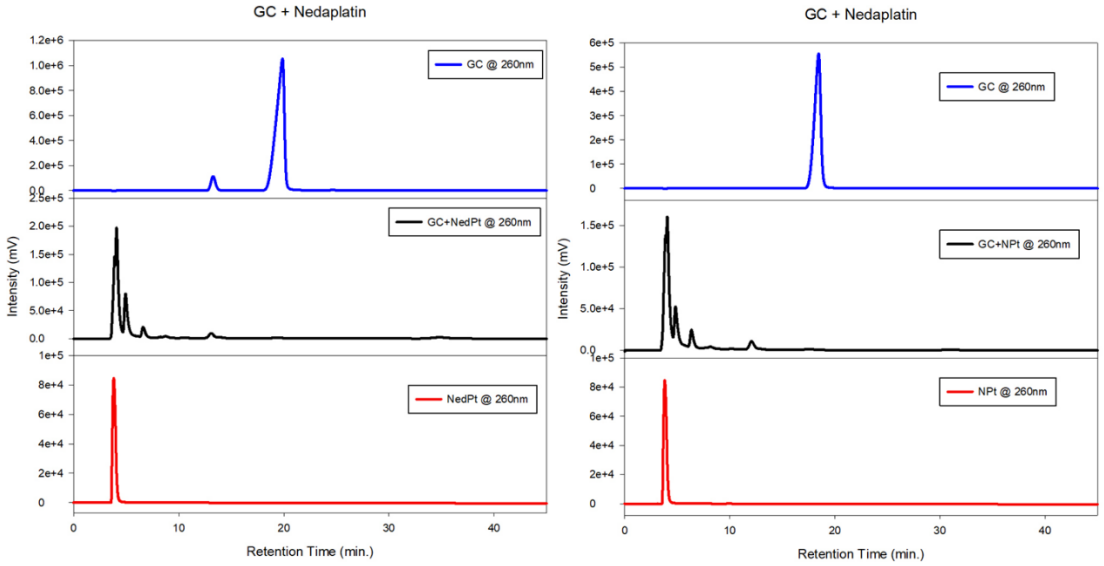


Figure 44. Illustrates two runs of GC DNA, nedaplatin, and GC+nedaplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

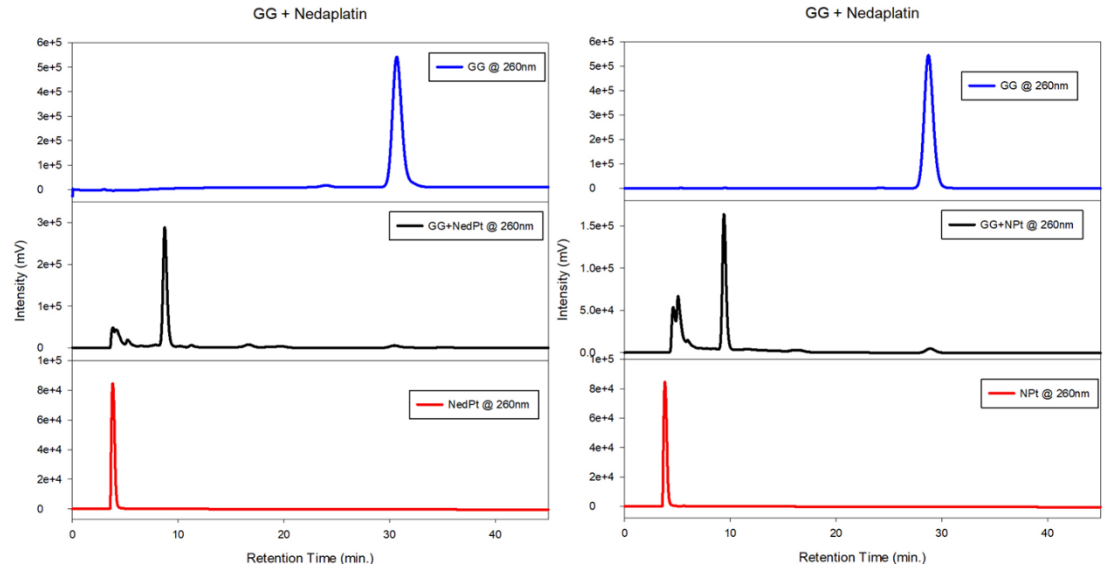


Figure 45. Illustrates two runs of GG DNA, nedaplatin, and GG+nedaplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

Table 15. Illustrates the calculations performed for HPLC data analysis. The calculations show the average peak areas for the individual DNA, the DNA peak(s) within the complex, and the % of DNA left unbound within the complex. The drugs are abbreviated carboplatin (Car), cisplatin (Cis), nedaplatin (Ned or N), and oxaliplatin (Ox or O). Each complex is shown with the DNA and the abbreviated drug (AA_N signifies AA DNA in a complex with nedaplatin).

	Area Under the Curve Calculations for DNA Complexes								
DNA Peak Areas (arb. units)		AA	AC	AG	CA	CG	GA	GC	GG
	run 1	33779407	30449737	29887987	29234149	28917872	43180184	61701948	33934769
	run 2	33423958	23411526	43647026	28705600	25882319	34052588	34052588	33200992
	Avg	33601683	26930632	36767507	28969875	27400096	38616386	47877268	33567881
DNA Peak Areas Within Complex (arb. units)		AA_N	AC_N	AG_N	CA_N	CG_N	GA_N	GC_N	GG_N
	run 1	1495562	834108	390541	489666	311250	952961	390529	441475
	run 2	76651	17375	241894	189086	5286	108786	0	305631
	Avg	786106.5	425741.5	316217.5	339376	158268	530873.5	195264.5	373553
% of DNA Left Unbound in Complex		AA_N	AC_N	AG_N	CA_N	CG_N	GA_N	GC_N	GG_N
		2.3	1.6	0.86	1.2	0.58	1.4	0.41	1.1

Table 16. Illustrates the calculations performed for HPLC data analysis. The calculations show the average peak areas for the individual drug, the drug peak(s) within the complex, and the % of drug left unbound within the complex. The drugs are abbreviated carboplatin (Car), cisplatin (Cis), nedaplatin (Ned or N), and oxaliplatin (Ox or O). Each complex is shown with the DNA and the abbreviated drug (AA_N signifies AA DNA in a complex with nedaplatin).

	Area Under the Curve Calculations for Drug Complexes								
Drug Peak Areas (arb. units)		Car	Cis	Neda	Ox				
	run 1	3437663	523116	1798236	18549237				
	run 2	3446879	453120	2307234	17845406				
	Avg	3442271	488118	2052735	18197322				
Drug Peak Areas Within Complex (arb. units)		AA_N	AC_N	AG_N	CA_N	CG_N	GA_N	GC_N	GG_N
	run 1	910124	1321824	1360244	997458	1221428	845118	1956461	899203
	run 2	1341531	1005398	865871	4750560	946000	1083538	2110615	1214663
	Avg	1125828	1163611	1113058	2874009	1083714	964328	2033538	1056933
% of Drug Left Unbound in Complex		AA_N	AC_N	AG_N	CA_N	CG_N	GA_N	GC_N	GG_N
		54.8	56.7	54.2	140.0	52.8	46.9	99.1	51.5

Like the cisplatin complexes, nedaplatin complexes were introduced to HPLC using a 1:1 ratio. Variations within the nedaplatin data are also present when comparing to the trend. GG+nedaplatin yields ~1.1% of unbound DNA, GA+nedaplatin yields ~1.4% unbound DNA, GC+nedaplatin yields ~0.4% of unbound DNA, and AG+nedaplatin yields ~1% of unbound DNA.

Oxaliplatin + DNA

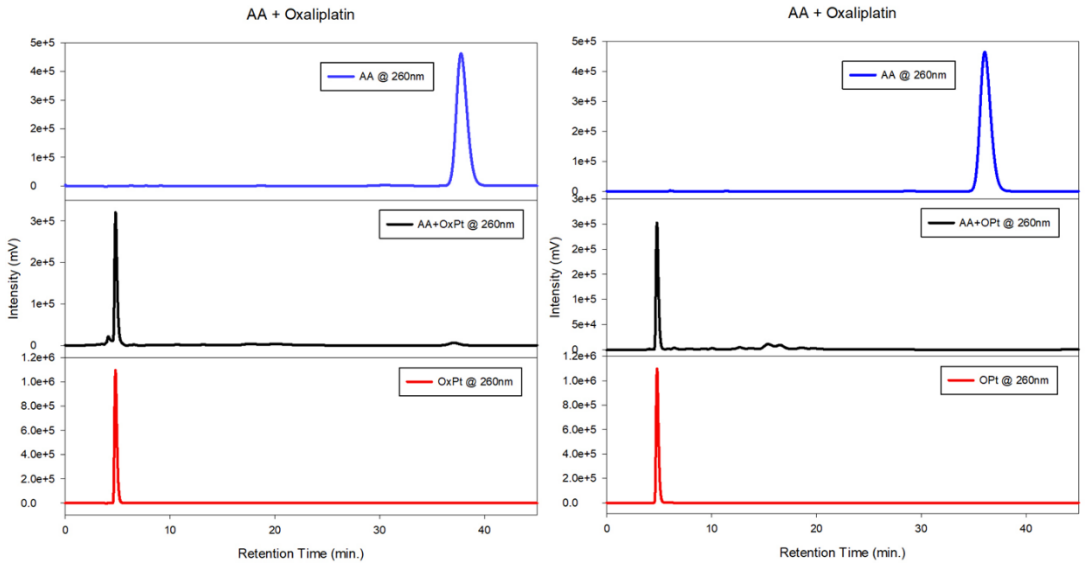


Figure 46. Illustrates two runs of AA DNA, oxaliplatin, and AA+oxaliplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

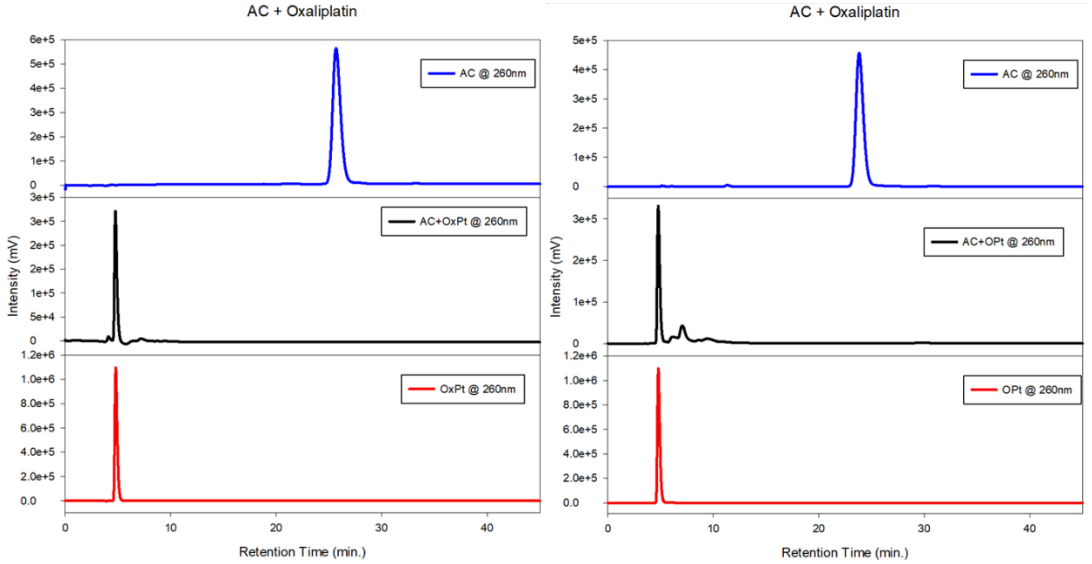


Figure 47. Illustrates two runs of AC DNA, oxaliplatin, and AC+oxaliplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

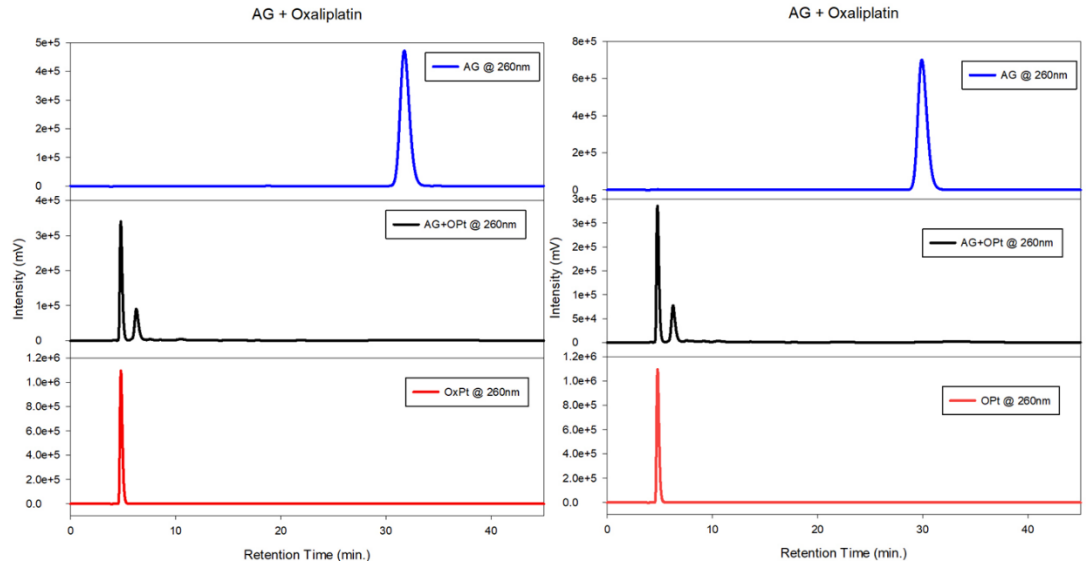


Figure 48. Illustrates two runs of AG DNA, oxaliplatin, and AG+oxaliplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

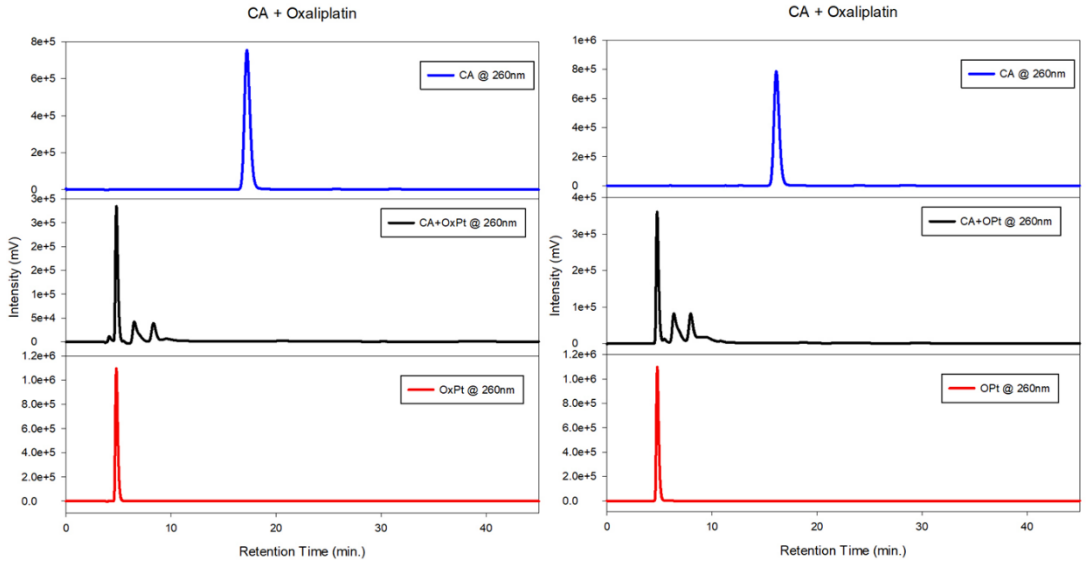


Figure 49. Illustrates two runs of CA DNA, oxaliplatin, and CA+oxaliplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

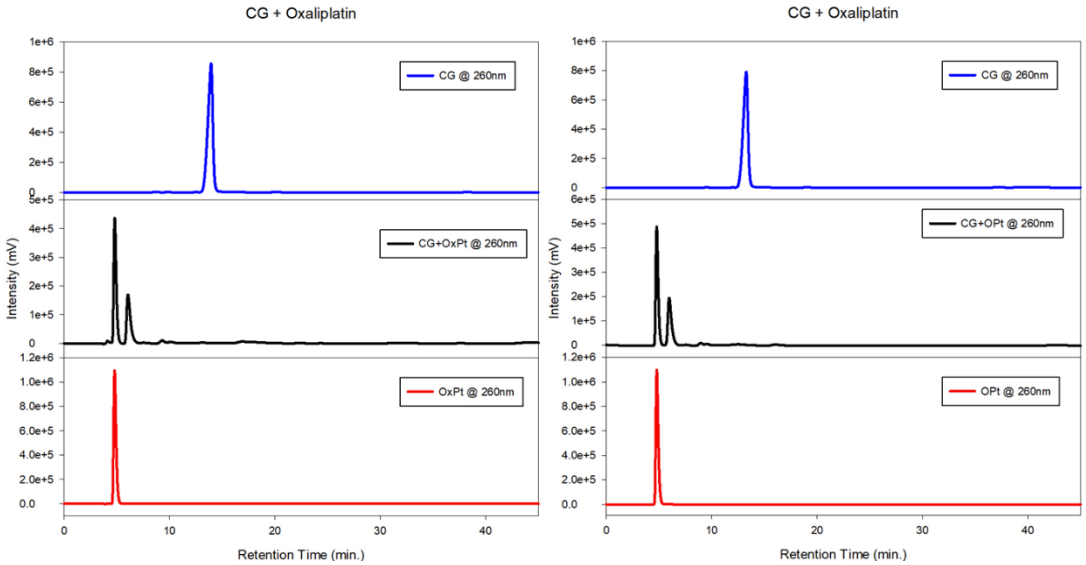


Figure 50. Illustrates two runs of CG DNA, oxaliplatin, and CG+oxaliplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

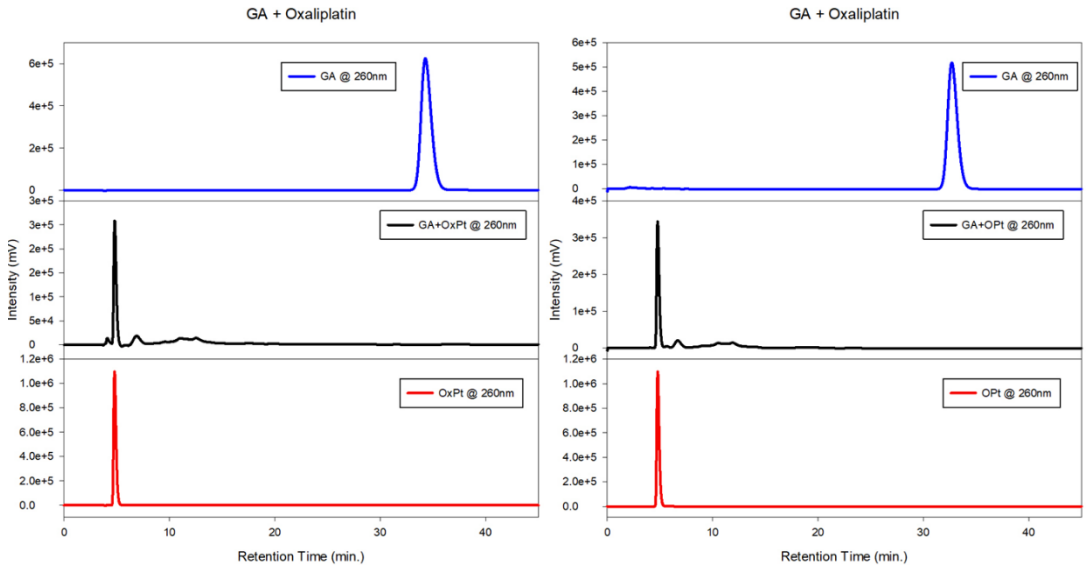


Figure 51. Illustrates two runs of GA DNA, oxaliplatin, and GA+oxaliplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

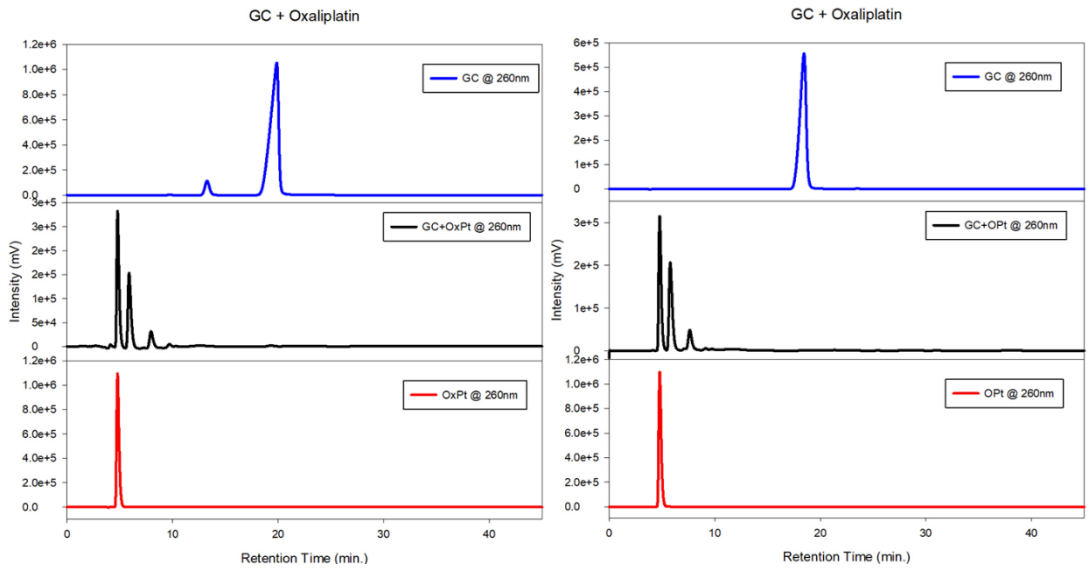


Figure 52. Illustrates two runs of GC DNA, oxaliplatin, and GC+oxaliplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

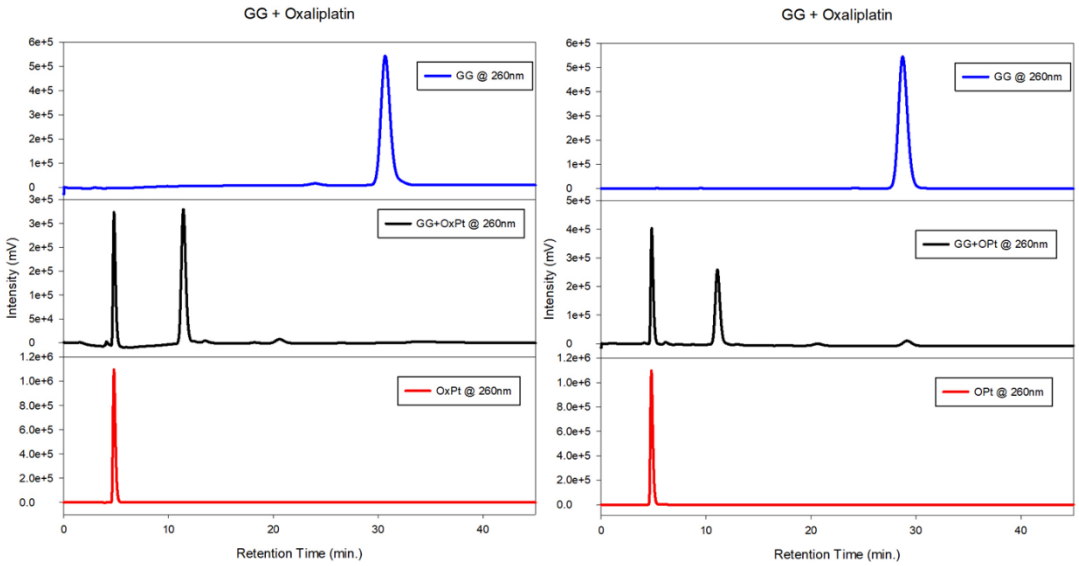


Figure 53. Illustrates two runs of GG DNA, oxaliplatin, and GG+oxaliplatin complex. All scans were ran using an 80:20 gradient of 2M TEAA and H_2O. (left) is Run 1 using one premade stock solution of 2M TEAA and (right) is Run 2 using a different premade stock solution of 2M TEAA.

Table 17. Illustrates the calculations performed for HPLC data analysis. The calculations show the average peak areas for the individual DNA, the DNA peak(s) within the complex, and the % of DNA left unbound within the complex. The drugs are abbreviated carboplatin (Car), cisplatin (Cis), nedaplatin (Ned or N), and oxaliplatin (Ox or O). Each complex is shown with the DNA and the abbreviated drug (AA_O signifies AA DNA in a complex with oxaliplatin).

	Area Under the Curve Calculations for DNA Complexes								
DNA Peak Areas (arb. units)		AA	AC	AG	CA	CG	GA	GC	GG
	run 1	33779407	30449737	29887987	29234149	28917872	43180184	61701948	33934769
	run 2	33423958	23411526	43647026	28705600	25882319	34052588	34052588	33200992
	Avg	33601683	26930632	36767507	28969875	27400096	38616386	47877268	33567881
DNA Peak Areas Within Complex (arb. units)		AA_O	AC_O	AG_O	CA_O	CG_O	GA_O	GC_O	GG_O
	run 1	420867	0	0	13279	130731	0	217279	18460
	run 2	0	0	0	0	0	14422	38592	1072532
	Avg	210433.5	0	0	6639.5	65365.5	7211	127935.5	545496
% of DNA Left Unbound in Complex		AA_O	AC_O	AG_O	CA_O	CG_O	GA_O	GC_O	GG_O
		0.63	0	0	0.02	0.24	0.02	0.27	1.63

Table 18. Illustrates the calculations performed for HPLC data analysis. The calculations show the average peak areas for the individual drug, the drug peak(s) within the complex, and the % of drug left unbound within the complex. The drugs are abbreviated carboplatin (Car), cisplatin (Cis), nedaplatin (Ned or N), and oxaliplatin (Ox or O). Each complex is shown with the DNA and the abbreviated drug (AA_O signifies AA DNA in a complex with oxaliplatin).

	Area Under the Curve Calculations for Drug Complexes								
Drug Peak Areas (arb. units)		Car	Cis	Neda	Ox				
	run 1	3437663	523116	1798236	18549237				
	run 2	3446879	453120	2307234	17845406				
	Avg	3442271	488118	2052735	18197322				
Drug Peak Areas Within Complex (arb. units)		AA_O	AC_O	AG_O	CA_O	CG_O	GA_O	GC_O	GG_O
	run 1	5826376	4730095	5762092	5036627	7612334	4532044	4706149	4885816
	run 2	4657188	5787914	4870503	6187931	8260704	5909256	5299962	6931945
	Avg	5241782	5259005	5316298	5612279	7936519	5220650	5003056	5908881
% of Drug Left Unbound in Complex		AA_O	AC_O	AG_O	CA_O	CG_O	GA_O	GC_O	GG_O
		28.8	28.9	29.2	30.8	43.6	28.7	27.5	32.5

Oxaliplatin complexes were synthesized at a 1:1 volume ratio similarly to cisplatin and nedaplatin complexes. Like all previous HPLC data presented, there are variations in the oxaliplatin complexes as well as the GG+oxaliplatin complex yields ~1.6% of unbound DNA, GA+oxaliplatin complex yields ~0.02% unbound DNA, GC+oxaliplatin yields ~0.27% unbound DNA, and AG+oxaliplatin complex yields ~0% unbound DNA.

Conclusion

RP-HPLC has exhibited many promising factors in analysis of DNA modification by platinum drugs. In each data set, there were visible spectral changes within each complex compared to when the drug and DNA were obtained separately. In most of the complexes, a significant peak arose leading to believe that an intermediate was produced, or a new product was formed. Another trend identified when comparing each complex across all drugs is the disappearance of the DNA peak within the complex with the exception of carboplatin data. The degradation of the DNA peak within these complexes leads to the assumption that the drug completely took over the DNA and destabilized it or that most of the DNA within the complex contributed to the new product or intermediate peak.

Table 19. **Is an overview of the DNA to Drug interactions based on the percentages of unbound DNA from HPLC data.** According to the overview - oxaliplatin interacted the most with each DNA, then cisplatin, then nedaplatin leaving carboplatin to interact the least with each DNA. Note that there was twice as much carboplatin in each complex, but still it converted the least amount of DNA.

DNA	*Platinum Drug Interaction (Based on Percentage of Unbound DNA)*							
AA	cisplatin 0.1%	>	oxaliplatin 0.6%	>	nedaplatin 2.3%	>	carboplatin 28.2%	
AC	oxaliplatin 0.0%	>	cisplatin 0.4%	>	nedaplatin 1.6%	>	carboplatin 21.0%	
AG	oxaliplatin 0.0%	>	cisplatin 0.2%	>	nedaplatin 0.9%	>	carboplatin 29.2%	
CA	oxaliplatin 0.03%	>	cisplatin 0.5%	>	nedaplatin 1.2%	>	carboplatin 28.8%	
CG	oxaliplatin 0.2%	>	cisplatin 0.4%	>	nedaplatin 0.6%	>	carboplatin 20.8%	
GA	cisplatin 0.01%	>	oxaliplatin 0.02%	>	nedaplatin 1.4%	>	carboplatin 16.5%	
GC	oxaliplatin 0.3%	>	nedaplatin 0.4%	>	cisplatin 1.9%	>	carboplatin 5.6%	
GG	cisplatin 0.08%	>	nedaplatin 1.1%	>	oxaliplatin 1.6%	>	carboplatin 15.8%	

A visual comparison of the efficiency of the drug when interacted with various DNA is shown below in **Figure 54**. It can easily be seen that carboplatin, even though the DNA to drug ratio was increased to 1:2, was the least effective drug when attempting to bind to DNA and decrease its stability.

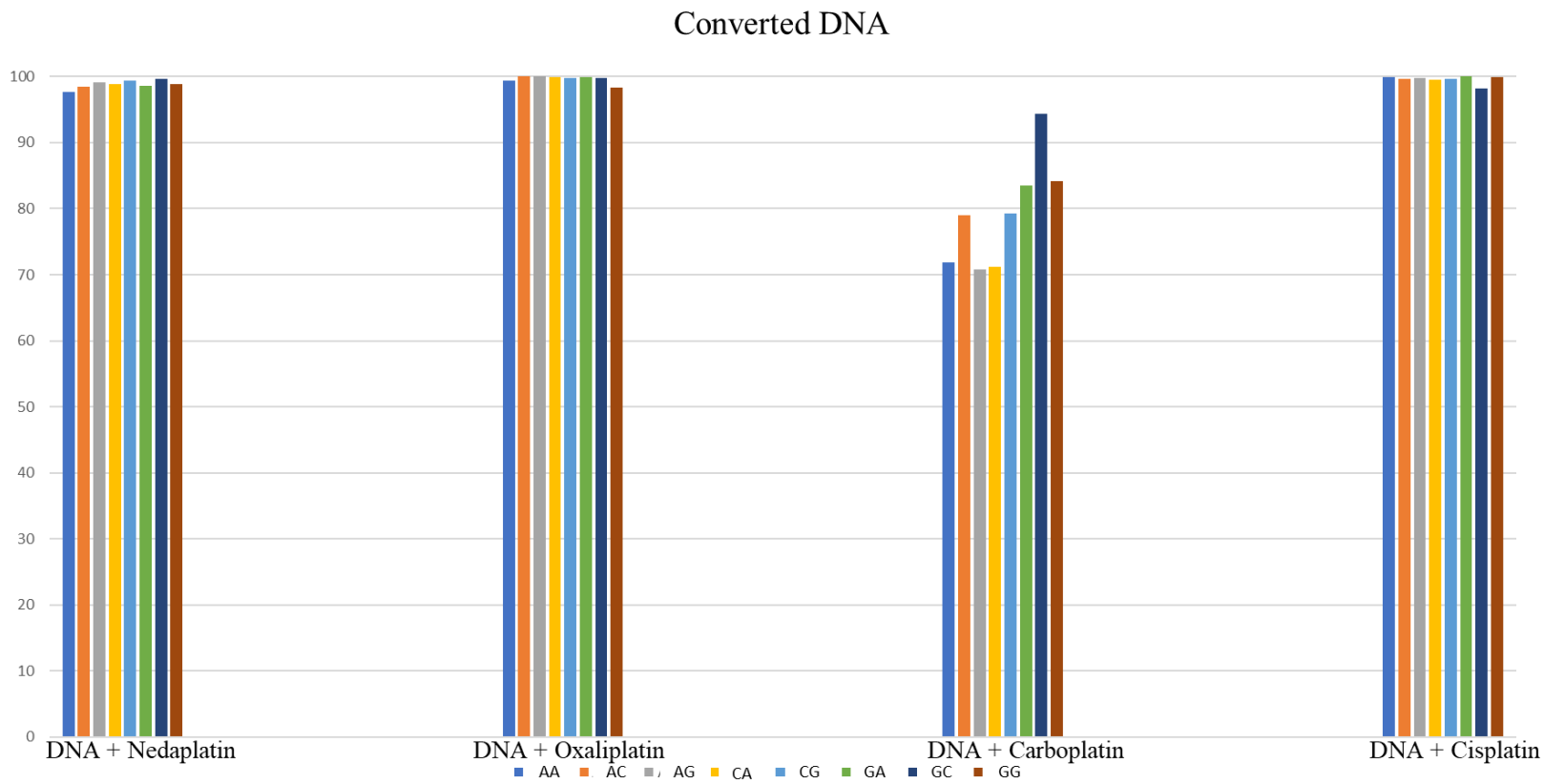


Figure 54. Is a comparison of the Platinum drugs and DNA interactions (from left: nedaplatin, oxaliplatin, carboplatin, and cisplatin). The plots illustrate the percentage of converted (bound) DNA to the platinum drugs. In the complexes with nedaplatin, oxaliplatin, and cisplatin almost 100% of each DNA was completely bound to the drug. However, in the carboplatin complexes, roughly 80% of the DNA was bound.

CHAPTER VII

CLOSING REMARKS AND FUTURE WORK

One of the main concerns for this research project was to see if the trend shown in the first chapter of this thesis, GG, GA, GC, and AG, remains true with all platinum drugs, or if it is just known for cisplatin. Even though eight DNA were interacted with platinum drugs, for the purpose of this trend, the following is a summary of the drug interactions with the four dinucleotides in the trend.

Table 20. Shows conclusions and trends found for the interactions between each DNA and drug complex.

DNA	Conclusions
GG	cisplatin interacted best with GG than nedaplatin and oxaliplatin, but cisplatin, nedaplatin, and oxaliplatin preferred to interact with GG more than carboplatin. Trend: cisplatin > nedaplatin > oxaliplatin > carboplatin
GA	cisplatin and oxaliplatin preferred to interact with GA more than carboplatin and nedaplatin. Trend: cisplatin > oxaliplatin > nedaplatin > carboplatin
GC	oxaliplatin and nedaplatin preferred to interact with GC more than cisplatin and carboplatin. Trend: oxaliplatin > nedaplatin > cisplatin > carboplatin
AG	oxaliplatin, cisplatin, and nedaplatin preferred to interact with AG more than carboplatin. Trend: oxaliplatin > cisplatin > nedaplatin > carboplatin

Summary of conclusions above

Out of all the drugs, carboplatin did not significantly modify the DNA

- carboplatin caused more than 5% of the DNA to be left unbound
- Less than 2% of the DNA was left unbound with the other drugs

Even though carboplatin complexes were made at a 1:2 volume ratio it left more DNA unbound compared to the other drug complexes which were ran at a 1:1 ratio

Averaging the drug interactions, cisplatin and oxaliplatin left less DNA unbound than nedaplatin did, but carboplatin in all experiments left significantly more DNA unbound.

Even though RP-HPLC has shown promise for the future analysis of platinum drugs and DNA complexes, the experimental procedures need to be altered to produce less variations for optimal results.

Comparing results from the HPLC and SERS experiments, in both, cisplatin modified GG the most. Throughout this research project several DNA were introduced to four platinum chemotherapeutic drugs and observed by SERS and HPLC, but in each experiment – carboplatin was shown to be the least effective based on data. In each case, carboplatin showed to have modified each DNA less than cisplatin as well as the other platinum drugs. Below is a table summarizing the trends found in both SERS and HPLC.

Table 21. Compares the trends found using both SERS and HPLC.

SERS Trends	HPLC Trends
AG: cisplatin > carboplatin	AG: oxaliplatin > cisplatin > nedaplatin > carboplatin
GA: cisplatin > carboplatin	GA: cisplatin > oxaliplatin > nedaplatin > carboplatin
GG: cisplatin > carboplatin	GG: cisplatin > nedaplatin > oxaliplatin > carboplatin

In the future, complex ratios are going to be synthesized using a molar ratio rather than a volume ratio to ensure all modifications are properly observed as they would in vivo. Incorporating HPLC into this research group is new so once the procedure is optimized, experiments need to continue to be repeated to check reproducibility to ensure proper analysis of the DNA to platinum drug complex interactions. This research project has aided in providing further information for understanding how platinum-based chemotherapeutic drugs modify DNA and affect its stability.

REFERENCES

1. Barhoumi, A.; Zhang, D.; Tam, F.; Halas, N. J. *JACS* **2008**, *130* (16), 5523–5529.
2. Potara, M.; Nagy-Simon, T.; Craciun, A. M.; Suarasan, S.; Licarete, E.; Imre-Lucaci, F.; Astilean, S. *ACS Applied Materials & Interfaces* **2017**, *9* (38), 32565–32576.
3. National Center for Biotechnology Information. PubChem Database. *Nedaplatin*, CID=72120, https://pubchem.ncbi.nlm.nih.gov/compound/Nedaplatin (accessed on Dec 2, 2019)
4. Shimada M, Itamochi H, Kigawa J. Nedaplatin: a cisplatin derivative in cancer chemotherapy. *Cancer Manag Res*. **2013**; 5:67-76. Published 2013 May 8. doi:10.2147/CMAR.S35785
5. National Center for Biotechnology Information. PubChem Database. *Lipoxal*, CID=43805, https://pubchem.ncbi.nlm.nih.gov/compound/oxaliplatin (accessed on Dec 2, 2019)
6. Choi CH, Cha YJ, An CS, et al. Molecular mechanisms of heptaplatin effective against cisplatin-resistant cancer cell lines: less involvement of metallothionein. *Cancer Cell Int*. **2004**;4(1):6. Published 2004 Oct 19. doi:10.1186/1475-2867-4-6
7. Desoize B, Madoulet C. Particular aspects of platinum compounds used at present in cancer treatment. *Crit Rev Oncol Hematol*. **2002**;42(3):317-325. doi:10.1016/s1040-8428(01)00219-0
8. Ji, L.; Sun, Y.; Xie, Y.; Wang, H.; Qian, H.; Wang, L.; Yao, W. *Vibrational Spectroscopy* **2015**, *79*, 44–51.
9. Jeanmaire, D. L.; Van Duyne, R. P. J. *Electroanal. Chem.* **1977**, 84, 1–20.
10. Lee, P. C.; Meisel, D. *J. Phys. Chem.* **1982**, 86, 17, 3391–3395, Publication Date: August 1, 1982, https://doi.org/10.1021/j100214a025
11. Souca, F. Graziele et al. Braz. J. Pharm. Sci. 2014;50(4)
12. Suzanne E. Sherman and Stephen J. Lippard, *Chemical Reviews* **1987** 87 (5), 1153-1181, DOI: 10.1021/cr00081a013
13. Johan Raber, Chuanbao Zhu, and Leif A. Eriksson, *The Journal of Physical Chemistry* B **2005** 109 (21), 11006-11015, DOI: 10.1021/jp050057d
14. Hicks, M.; Wharton, G.; Huchital, D. H.; Murphy, W. R.; Sheardy, R. D. Assessing the Sequence Specificity in the Binding of Co(III) to DNA via a Thermodynamic Approach. *Biopolymers* **1997**, 42 (5), 549–559.
15. K. Lance Kelly, Eduardo Coronado, Lin Lin Zhao, and George C. Schatz, *The Journal of Physical Chemistry* B **2003** 107 (3), 668-677, DOI: 10.1021/jp026731y
16. *The Optical Properties of Metal Nanoparticles*: https://pubs.acs.org/doi/10.1021/jp026731y (accessed Sep 12, 2019).
17. Libretexts. 4.3: Raman Spectroscopy. https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Book:_Physical_Methods_in_Chemistry_and_Nano_Science_(Barron)/04:_Chemical_Speciation/4.03:_Raman_Spectroscopy (accessed Aug 23, 2019).
18. He, L.; Lin, M. Application of surface enhanced Raman spectroscopy to food safety issues. dissertation, **2009**.

19. Raman Spectroscopy and Raman Microscopy: Thermo Fisher Scientific - US. https://www.thermofisher.com/us/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/raman-spectroscopy.html (accessed Jun 4, 2018).
20. Koglin, E.; Séquaris, J.-M.; Fritz, J.-C.; Valenta, P. *J. Mol. Structure* **1984**, *114*, 219–223.
21. Otto, C.; T. J. J. Van Den Tweel; Mul, F. F. M. D.; Greve, J. Surface-Enhanced Raman Spectroscopy of DNA Bases. *Journal of Raman Spectroscopy* **1986**, *17* (3), 289–298.
22. Östblom, M.; Liedberg, B.; Demers, L. M.; Mirkin, C. A. *J. Phys. Chem. B* **2005**, *109* (31), 15150–15160.
23. Skoog, D. A.; Holler, F. J.; Nieman, T. A. *Principles of Instrumental Analysis*; 5th ed.; Wadsworth Publishing Co Ins: **1997**.
24. Lesson 1. Basic of Raman scattering. https://www.nanophoton.net/raman-spectroscopy/lessons/lesson-1 (accessed Feb 24, 2020).
25. Johnstone, T. C.; Suntharalingam, K.; Lippard, S. J. The Next Generation of Platinum Drugs: Targeted Pt(II) Agents, Nanoparticle Delivery, and Pt(IV) Prodrugs. Chemical Reviews **2016**, 116 (5), 3436–3486.
26. Östblom, M.; Liedberg, B.; Demers, L. M.; Mirkin, C. A. On the Structure and Desorption Dynamics of DNA Bases Adsorbed on Gold: A Temperature-Programmed Study. *J. Phys. Chem.* B **2005**, 109 (31), 15150–15160.
27. Yost, R. W.; Ettre, L. S.; Conlon, R. D. Practical liquid chromatography: an introduction; Perkin-Elmer: Norwalk, CT, **1980**.
28. Harrington, C. F.; Pla, R. C. L.; Jones, G. D. D.; Thomas, A. L.; Farmer, P. B. Chemical Research in Toxicology **2010**, 23 (8), 1313–1321.
29. Kozelka, J. Inorganica Chimica Acta **2009**, 362 (3), 651–668.
30. Baik, M.-H.; Friesner, R. A.; Lippard, S. J. Theoretical Study of cisplatin Binding to Purine Bases: Why Does cisplatin Prefer guanine over adenine? Journal of the American Chemical Society **2003**, 125 (46), 14082–14092.
31. Jess, P. R.; Smith, D. D.; Mazilu, M.; Dholakia, K.; Riches, A. C.; Herrington, C. S. Early Detection of Cervical Neoplasia by Raman Spectroscopy. *International Journal of Cancer* **2007**, 121 (12), 2723–2728.
32. Xu, L.-J.; Lei, Z.-C.; Li, J.; Zong, C.; Yang, C. J.; Ren, B. Label-Free Surface-Enhanced Raman Spectroscopy Detection of DNA with Single-Base Sensitivity. *JACS*, **2015**, 137 (15), 5149–5154.
33. Sun, K.; Huang, Q.; Meng, G.; Lu, Y. Highly Sensitive and Selective Surface-Enhanced Raman Spectroscopy Label-Free Detection of 3,3′,4,4′-Tetrachlorobiphenyl Using DNA Aptamer-Modified Ag-Nanorod Arrays. *ACS Applied Materials & Interfaces* **2016**, 8 (8), 5723–5728