Vitamin-Enriched Pharmachar: A Renewable Carbon-Based Organocatalyst

By

Andrew Gracie

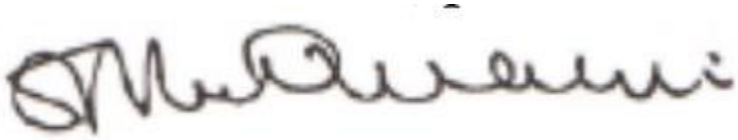
A thesis submitted in partial fulfillment of the requirements for the degree of

Bachelor of Science Honours (Chemistry)

Cape Breton University

Approved by

Dr. Stephanie MacQuarrie, PhD, Department of Chemistry, Cape Breton University



Dr. Geoffrey Lee-Dadswell, PhD, Department of Math Physics Geology, Cape Breton University

Ms. Judy MacInnis, BEd, MSc, Department of Chemistry, Cape Breton University

Abstract

The demand for medications has been growing non-stop and this subsequently results in an increase in the amount of pharmaceutical waste being generated year over year. This pharmaceutical waste makes its way into the environment via disposal from manufacturing plants as well as the typical household. The current method of dealing with this waste is through incineration, which tends to release harmful emissions into the atmosphere while also creating ash that is disposed of into landfills, with no valuable application available. To mitigate environmental pollution, as well as create valuable products from this waste material, the process of pyrolysis is employed.

With pyrolysis, no ash is produced and instead we get products with potential applications to help the environment rather than harm it. These products, when generated from pharmaceuticals, are denoted as pharmachar, pharmaoil, and pharmagas. Pharmachar is similar to ash, but it has both surface area and porosity which can lead to various applications across a wide range of industries through the process of sorption.

This thesis focuses on pharmachar and the adsorption of a vitamin, riboflavin, which has been shown to participate catalytically in photo-redox reactions via a single electron transport mechanism. The goal of this study is to create a renewable and heterogenous carbon based support for this organocatalyst molecule and to investigate the parameters of adsorption, with the future work to assess the photo-catalytic activity of the pharmachar-riboflavin complex.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AcetaChar – Acetaminophen-based BioChar

APIs – Active Pharmaceutical Ingredients

BET- Brunauer-Emmett-Teller

FAD – Flavin Adenine Dinucleotide

FMN – Flavin Mononucleotide

FTIR- Fourier Transform Infrared (Spectroscopy)

KBr – Potassium Bromide

OTC – Over the Counter

PharmaChar – General term for pharmaceutical-based Biochar

TEM – Transmission Electron Microscope

UV-Vis – Ultraviolet-visible (light)

1 Introduction

1.1 Pharmaceutical Waste

Each year, the pharmaceutical industry continues to see growth, in fact, in 2021 over-the-counter (OTC) medication comprised $3.7 Billion of all pharmaceutical sales in Canada alone1. With this industry facing unending demand, we see more waste being dumped into the environment each year from manufacturing, hospital, and patient disposal[2–4].

Medical waste is a very broad term with no definitive consensus on what medical waste is; this makes dealing with these kinds of waste even more cumbersome, especially when comparing countries with different standards. Each nation designates waste slightly differently; however, it is usually broken up into two categories: general and hazardous. In 2018, the World Health Organization (WHO) published a newsletter regarding healthcare waste. They estimate that ~15% of all medical waste generated is hazardous to some degree; whether it is microbial, chemical or even radioactive[5]. Even then, the true amount of hazardous waste generated could still be higher, as many countries that do not have sufficient funding or protocols for hazardous and non-hazardous waste together[5,6]. In contrast, countries with high GDP per capita such as the, U.S.A typically have good disposal practices yet generate significantly more waste. For example, a review on Medical Waste Management authored by Windfeld and Brooks, presented waste production metrics of various nations from a range of studies, and the U.S.A was shown to produce 10.7 kg/bed·day of healthcare. In contrast, Tanzania produced 0.14 kg/bed·day of waste[7].

While efforts should be made to mitigate the amount of waste production and improper disposal, this is and will continue to be an issue for the global market. Most of the waste generated is from the production process, however, billions of people worldwide consume medications daily. This leads to unintended disposal into the waste stream through the normal bodily excretory process and disposal of expired or simply unused medication via the garbage or drain[3,4,8,9]. These medications often end up as toxic sludge at sewage and waste treatment plants. This matrix contains many active pharmaceutical ingredients (APIs) ,which, if let into the environment, could pose significant human health risks, especially related to antibiotic resistance[6,9,10].

1.1.1 Incineration of Waste Pharmaceuticals

In recent decades there has been an increasing need for treating and properly disposing of pharmaceutical waste. The main method for waste treatment has been to incinerate the waste. Incineration is heating or burning material to high temperatures (>1000°C) to cause combustion reactions which expels large quantities of atmospheric pollutants[7,11]. The goal is to achieve complete combustion to produce only CO_2 and H_2O. However, that is the highly ideal and very rare case with incineration, especially as it relates to complex mixtures of waste[11]. This not only produces harmful emissions, such as: poly-aromatic hydrocarbons (PAHs), dioxins, furans, and compounds containing heavy metals; it also produces residual solid waste. This residual waste, ash, while significantly reduced in weight and volume, still poses a potential for the leaching of toxic substances into the landfill where they are dumped. There is the possibility to re-capture the emissions of these incinerators using various adsorbents. However, there is no value to the solid waste produced and it still poses an issue to the environment. Other methods of “treating” pharmaceutical waste are simply direct land filling/composting[12].

Rather than continuing this cycle of turning garbage into more garbage, researchers have been interested in other emerging methods of waste treatment that produce lower emissions and or inert waste products with the potential to be used in various applications.

1.2 Pyrolysis and Applications of Pharmaceutical Waste

Like incineration, pyrolysis is heating a substance to a high temperature while under an inert atmosphere, to prevent compete combustion and ashing[13–15]. The major products of this reaction are oils, which may have potential as a renewable energy source or high value small molecule production, as well as char and gas vapors (which can be re-captured via adsorption/carbon sequestration).

Research in this area is limited, with only a few examples in the literature. In 2020 Chen and co-workers showed that clean energy could be produced by co-pyrolysis of waste pharmaceutical sludge and biomass generated from Ginkgo Biloba, a tree native to China, and the overall char made from this mixture had a reduced toxicity[12].

Li et. al. showed that char derived from pharmaceutical sludge could be a low-cost raw material for preparing adsorbents to remove tetracycline from water. They showed that pharmaceutical sludge char generated at 600 °C could absorb 157.38 mg/g of tetracycline at 25 °C and determined that adsorption was via both physisorption and chemisorption[9].

Zhang and co-workers used iron and nitrogen-rich pharmaceutical sludge char for rapid pollution degradation. They demonstrated the active sites could be tailored based on temperature and pyrolysis conditions[16].

Thus far, every example from the literature implements sludge as the starting pharmaceutical waste; to the best of our knowledge no work has been done in this area detailing the impacts of

the presence of specifics drugs within the waste streams and how the char, oil, and gas may be impacted. The MacQuarrie group is carrying out this systematic study looking at the char properties based on specific pharmaceuticals. This work will focus on the char created from the pyrolysis of a single pharmaceutical – acetaminophen, and applications of that PharmaChar.

1.3 Acetaminophen Properties, Prevalence, and Waste

Acetaminophen is the first candidate to examine because it is readily available, highly consumed, and has a simple molecular structure. Roughly 60 million people in the U.S. alone consume acetaminophen in a given week[17]. When consumed, approximately nine percent of the drug passes through the body un-metabolized, resulting in Acetaminophen in wastewater[18]. While there are efforts to treat this wastewater before it makes its way to the environment, the high consumption rate means there is a steady supply of acetaminophen in wastewater with no signs of slowing down given how popular this analgesic is.

Acetaminophen can be quite toxic to adults in high enough doses (> 4000 mg of active ingredient per day), particularly to the liver[17,18]. This emphasizes the need not only to treat contaminated water, but also find other methods of wasting expired or unused acetaminophen tablets before they are tossed in the waste bin and sent to a landfill, in where they could degrade and leach into the water table[19].

The acetaminophen structure can be seen in Figure 1 below; as the name suggests, we see an acetyl-amide functional group attached to a phenol moiety. This structure is aromatic, which, along with the long chain sugar and starch molecules that make up the filler components of the OTC medication, should provide a good basis for the generation of Char, which consists of many

aromatic rings all condensed to form what is essentially graphene sheets, coated with various functional groups.

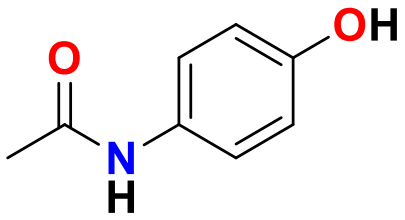


Figure 1: Acetaminophen Molecule

Previous unpublished work from the MacQuarrie group has shown that PharmaChar created from acetaminophen, is carbon-rich, hydrophobic, and contains alcohol groups, as does the parent structure. This previous, and on-going work is being collected in a mass database; however, this project focuses on the applications of chars created from acetaminophen (AcetaChar). This thesis will focus on investigating AcetaChar as an adsorbent for riboflavin.

1.4 Riboflavin

Riboflavin, a water-soluble isoalloxazine derivative with a ribitol group attached, is commonly known as Vitamin B2. First derived from milk whey in the late 1800s, it is now widely known to be vital to the human body and imperative that it is consumed in our diets[20].

Riboflavin, typically in the bio-active forms, flavin-mononucleotide (FMN) or flavin adenine dinucleotide (FAD) in the body, facilitates oxidation-reduction (Redox) reactions for various biochemical and physiological mechanisms. This molecule and its multiple derivatives, take up many roles in the human body. Still, most notably, the principal part is in managing both the 2-electron acceptor/donor and the 1-electron acceptor/donor complexes of the electron transport chain[20,21].

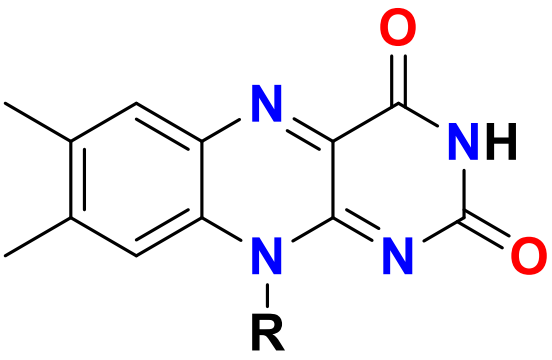


Figure 2: Isoalloxazine Molecule

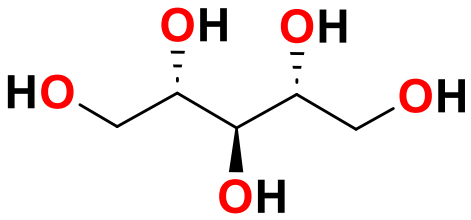


Figure 3: Ribitol Molecule

Heavy metals such as Palladium and Iridium are known to have excellent catalytic activity and other smaller metals like iron are used as co-factors for various enzymes; however, recovery of these metals from reactions can be cumbersome along with the fact that these metals are typically quite toxic to humans. There is considerable interest then, in organic (carbon-based and metal-free) catalysts.

One such organo-catalyst is Riboflavin, as mentioned above, riboflavin can participate in oxidation reduction reactions in the body which means this can be taken advantage of for lab scale reactions as well. Riboflavin, an inexpensive and non-toxic vitamin, can participate in photocatalytic (light-activated) reactions via a single-electron transfer mechanism and this was shown in reactions transforming bi-aryl carboxylic acids to 3,4-benzo-coumarin molecules, which are used for the production of various anti-bacterial and antifungal medications[22].

Riboflavin has been adsorbed on biochars for waste-water treatment as, even though riboflavin is considered to be non-toxic, it contains a large quantity of nitrogen atoms and so when this molecule breaks down in the water-supply it has the potential to contribute to eutrophication[23].

Adsorption of riboflavin is also of interest regarding the production of riboflavin. Riboflavin cannot be made by the human body, but it can be made by certain microbial species. In this bio-fermentation process however, the product is not pure riboflavin and so the mixture has to be separated in order to purify the riboflavin content to then allow it to be sold as a vitamin for human consumption[24].

The study performed by Ma et. al. used commercially available carbon-nanotubes and magnetic carbon nanotubes as adsorbents for waste-water treatment and the work conducted by Liu and co-workers used biochar derived from algae to separate and there-by purify riboflavin from the bacterial fermentation process. To present date, and to the best of our knowledge, no work has been done to adsorb riboflavin using pharmachar along with the use of char to provide a heterogenous support for photocatalytic activity.

To fully take advantage of the pharmachar loaded with riboflavin, we must understand the mechanism of adsorption.

1.5 Sorption

Sorption is the process of either absorbing or adsorbing a substance, typically from a liquid to a certain type of solid material. Absorption refers to the substance (absorbate) being taken up into the bulk phase of the solid (absorbent), like a sponge soaking up water molecules into its

pores. Whereas adsorption refers to some adsorbate molecule sticking or adhering to the surface of some adsorbent material; think dust sticking to the walls in a house.

Previous work from the MacQuarrie Group has shown that PharmaChar does not contain much, if any, porosity and so any sorption that occurs is likely to be entirely adsorption-based.

Adsorption occurs via two main mechanisms: physisorption and chemisorption. Chemisorption refers to the formation of chemical bonds (covalent or ionic) and so this process not only requires high temperatures and/or pressures but is generally considered irreversible. Chemisorption is not of interest given that the scope of this project is centred on creating a renewable adsorbent material. With physisorption then, adsorbate molecules collide with the surface of the adsorbent and adhere to the surface via van der Waals interactions (dipole-dipole forces, $\pi - \pi$ stacking, and hydrogen-bonding) and this can generally occur at room temperature. Since no chemical bonds are formed in this mechanism, the process is reversible which provides the ability to recycle and reuse these adsorbent materials[25].

1.6 Research Objectives

Pharmaceutical pollutants pose significant risks to both aquatic habitats and human health[2,4,6]. Adsorption of wastewater contaminants such as APIs has been of considerable interest amongst those researching biomass-based adsorbents. To date, there has yet to be any work looking into char made from commercial pharmaceuticals themselves as an adsorbent material. The scope of this project is centred on two central questions: is acetaminophen based PharmaChar a suitable adsorbent for Riboflavin in water, with the final goal of creating a carbon-based Organocatalyst?

2. Materials and Methods

2.1 Chemicals Used

Expired pharmaceuticals used were supplied by UnBound Chemicals, St. John's, Newfoundland. Bio-reagent grade Riboflavin (Vitamin B2) was purchased from Millipore Sigma. 95% Ethanol used for Char washing was purchased from Fischer Scientific. Ultrapure water, used in all solutions, was obtained from a Millipore Simplicity Ultrapure Water Dispenser. Fourier-transform-infrared-spectroscopy (FT-IR) grade KBr was purchased from Fischer Scientific and used to make KBr pellets in FT-IR characterization. A Nicolet© Summit FT-IR instrument with an ID7 transmission attachment was used in the FT-IR procedure, and a BMG Labtech Micro-plate Reader (in fluorescence mode) with a non-fluorescent NUNC 96-well micro-plate was used for all adsorption studies.

2.2 Pyrolysis Procedure

The pyrolysis reaction was conducted in a modified Thermocraft reactor (Figure 4). Commercially branded acetaminophen tablets were used for this project. The pill formulation is branded under Equate©, containing 500 mg of active ingredient per pill and a mix of sugars, starches, and non-medicinal chemical agents/dyes[26].

Before pyrolysis, the pills were pulverized (Figure 5) using a mortar and pestle until a powder consistency was reached. This powder is transferred to a small, clean metal can; this can is subsequently transferred to a larger, closed-off metal can, which has been modified to attach a condenser at the bottom. There is also a gas inlet at the top for the nitrogen sweep gas to enter. Once the apparatus was ready, the can is enclosed inside the reactor using an insulating material, then cold water was flushed through the condenser and the nitrogen gas is set to flow at 100

ml/min. As for the pyrolysis conditions, a heating ramp of 5.5°C/min was employed until reaching various final temperatures and holding there for 30 minutes. This was based on pyrolysis conditions of other waste materials[10,12,16,27]. PharmaChars are not well-studied in the literature, so this process is still in development stage. The holding temperature investigated was 800°C. Overall, the yield of BioChar from these pyrolysis experiments was ~23% of the original mass pyrolyzed.

Figure 4: Modified Thermocraft Pyro-Reactor

Figure 5: Equate Acetaminophen Tablets, Pulverized

2.3 N_2 Physisorption Procedure

Nitrogen physisorption analysis was performed using a Micromeritics ASAP 2020 Sorptometer. For N_2 physisorption, only a tiny portion of the adsorbent material is required. The first step for measuring N_2 physisorption was to weigh out the sample. For our instrument, a typical sample required is anywhere from 0.1000 g to 0.1500 grams. First, 0.1594 g of the pristine AcetaChar sample was weighed out using an analytical balance. No chemical or washing treatment was done to this sample. This portion of char was set aside and then the empty sample tube was weighed, with the cap attached. After weighing ten times and noting the average weight, 36.4236 g, the previously weighed char sample was then added to the sample tube. Next, this tube was attached to a de-gassing port and a heating mantle was clipped onto the bottom. The de-gassing conditions on the instrument was set and the sample was allowed to de-gas overnight. After allowing the sample to de-gas, both liquid nitrogen dewars on the instrument were filled to ensure the sample and reference tubes were kept at 77K during analysis. The sample tube was then weighed another 10 times with the average weight 36.5607 g being noted. This is what's known as the de-gassed sample tube weight and is essential in the accurate determination of B.E.T surface area. The empty tube and de-gassed sample tube weight are then input into the Sorptometer application, and a de-gassed sample weight of 0.1371 g was calculated. This is the weight of the sample after any volatiles have been expelled from the pores of the material. The sample tube was then attached to the analysis port and the conditions were set for N_2 Physisorption at 77K. Analysis was allowed to run overnight and the resulting data was collected the following morning.

The same procedure was conducted for the washed AcetaChar sample as well, which had a de-gassed sample weight of 0.1375 g.

2.4 Washing Procedure

After pyrolysis, the char will still contain organic molecules along the surface and in the pores. This is because in the reaction vessel, not all vapours are able to travel to the condenser and condense into the bio-oil, instead they adsorb into the char as it is being formed. The washing procedure involves using a Soxhlet extractor (Figure 6), to continuously rinse the char with fresh solvent, for multiple hours at a time. A solvent mixture of 60% ultrapure water (H_2O) and 40% Ethanol was chosen to remove both non-polar and polar compounds from the char. In any case, this washing step could use improvement as the energy needed to heat the solution along with the amount of water being flushed through the condenser is wasteful yet needed for the reasons stated above.

To determine if substances are leaching out of the char and into the solution, a UV-Visible Spectrophotometer is used to analyze the wash solution after the washing is complete; a Shimadzu UV-2600 instrument was used for char washing analysis. Each wash was performed using a 500ml round bottom flask, a Soxhlet extractor and thimble, Soxhlet condenser, a heating mantle, and a 120-volt variable transformer. The extractor was filled with 250 mL of solvent until it was siphoned into the round bottom, with an extra 5.0 ml to ensure the round bottom would not go dry. The char was loaded into a glass thimble with a cellulose filter on the bottom, this thimble was then placed inside the extractor as shown in Figure 6.

Figure 6: Soxhlet Apparatus

2.5 TEM Procedure

A Hitachi© 7700 Transmission Electron Microscope (TEM) was used to obtain images of the Char material on the nanometer scale (~10-300nm field of view). This instrument consists of a high-voltage (kV) electron gun located at the top of the microscope column; this electron gun uses a lanthanum hexaboride (Lab6) filament to produce a high energy beam of electrons which will be focused on the sample using various condenser lenses and an aperture. After passing through the sample the beam encounters a set of objective lenses along with another aperture and this help to focus the beam onto a fluorescent screen and camera, which subsequently produces the image on the computer screen[28]. The TEM images were used to determine if there were any differences before and after the adsorption of riboflavin onto acetaminophen char. Sample preparation involved taking several milligrams of sample into a microcentrifuge tube and adding in

0.5 mL of Ethanol, this was then sonicated for 15 minutes to fully disperse the sample throughout the ethanol. After the 15 minutes, 4 μL of the suspension was drop casted onto the 3 mm Copper grids which hold the sample in the instrument. The grids were 200 nm mesh copper (Cu) grids that were coated with carbon and Formvar ®.

2.6 Fourier Transform – Infrared Spectroscopy (FTIR)

In all cases in which this technique is used, the same procedure was followed. The method used in this procedure is the potassium bromide (KBr) pellet method for solid samples. First, a blank KBr pellet was made which consists of no sample. This pellet was then run on the Nicolet Summit FTIR instrument to produce what is known as the background spectra; this spectrum accounts for the contribution of KBr to the signal provided to the instrument. To create the blank pellet, a sample of analytical grade KBr is weighed out to be in the range of 0.2800 – 0.3000 g. This portion of KBr was then ground to a fine powder in an agate mortar and pestle.

After grinding, the sample was transferred to a KBr die. This die consists of two main pieces, a top and bottom. The bottom has a vacuum tube adapter, and the top consists of a hollow tube in which the sample is held. In this hollow tube, two flat pieces of steel, known as anvils, hold the sample in place and a steel rod known as a plunger is inserted on top of the superior anvil.

This assembled die with the KBr blank inside was then transferred to a hydraulic press. Once on the press's stage, the stage was raised by hand pumping until the plunger just touched the top of the press and the stage was then locked into place. A rubber tube (thick-walled) was then attached to the bottom piece of the KBr die via the vacuum adapter; this tube is attached to a vacuum pump which was subsequently turned on and the sample was evacuated for five minutes to pull out any excess moisture. After evacuation, the press was then pumped by-hand until the

pressure gauge reaches 20,000 pounds per square inch. This was left at this pressure for ten minutes. The vacuum pump was turned off and the stage locked was released slowly so as gradually lower the pressure from 20,000 psi back down to zero. Once the pressure was fully released, the KBr die was removed, and the blank pellet was put aside in a desiccator while the sample pellet was prepared.

In forming a sample pellet, the same procedure was followed as was done for the blank. The only difference is once the KBr has been ground, a small amount, ≈ 1 milligram (mg), of sample is then added to the mortar and the mixture is ground at least three more times to ensure homogeneity. If the sample is a fine powder, one to two milligrams will suffice; however, if the sample is coarse and or has an intense color, as is the case with char material, then it may be better to opt for a few grains of sample.

FT-IR characterization was performed for pure riboflavin, crushed Equate© Extra-Strength Acetaminophen tablets, acetaminophen-char (AcetaChar), and acetaminophen-char with riboflavin adsorbed. The respective infrared spectra are shown in Figures 17 and 18.

Analysis was then carried out using ID7 FTIR attachment for the Nicolet Summit FTIR instrument. The spectra were examined to detect any differences in the FTIR spectra and to ultimately see if any distinct functional groups for Riboflavin would appear in the riboflavin-adsorbed char. The resolution for this instrument was set to 4 cm^{-1} and 16 scans were taken for each spectrum.

2.7 Elemental Analysis

A sample of pristine AcetaChar was sent away for Elemental Analysis. Elemental analysis involving the elements carbon, hydrogen, and nitrogen (CHN analysis) was conducted at Western University using an Elementar Analyzer (Vario-Isotope Cube Model).

2.8 Adsorption Study

All adsorption studies were conducted at room temperature, in triplicate, using a G10 Gyro-Rotary Shaker and ultrapure H_2O as the solvent. To perform kinetics and isotherm modelling experiments, batch adsorption studies must be conducted under various conditions. This was done to optimize the parameters of adsorption so that it was possible to calculate the maximum possible adsorptive capacity of the AcetaChar for Riboflavin. With this information it was then of interest to work in the dynamic range of the adsorption to investigate the kinetics and isotherm models. The parameters investigated were adsorbent dose, contact time, and initial adsorbate concentration.

Stock Solution: Riboflavin stock solution (51.8mg/L) was prepared by weighing 0.0259 grams (25.9 mg) of riboflavin, transferred to a 500.0 ml volumetric flask, and then dissolved in ultrapure H_2O, and diluted to the calibration line.

Adsorption Experiment: Testing Adsorbent Amount. The procedure for the adsorbent dosage experiment involved using a Mettler-Toledo analytical balance to measure out four aliquots of each char amount, i.e., four aliquots each of 100.0, 200.0, 300.0, 400.0, and 500.0 mg. Each aliquot of char was added to a labelled 5.0 ml mini-centrifuge tube. One aliquot in each set of four was reserved to be a method-blank, used to correct for any contribution of the solvent and or char material to the signal that is produced in the fluorescent plate-reader. To each method-blank, 1000 μL or 1.000 ml of ultrapure H_2O was added via a 1000 μL micro-pipette. Then, in each of

the three aliquots for the various char amounts, 1000 μL or 1.000 ml of the 51.8 mg/L riboflavin stock was transferred to the centrifuge tubes, using the same 1000 μL micropipette. The four aliquots for each parameter were placed into separate, labelled, 250.0 ml Erlenmeyer flasks which were then placed on a G10 Gyro-Rotary shaker and were allowed to stir for four hours at room temperature and a rate of 150 revolutions per minute (rpm). After the samples completed shaking on the Gyro-Rotary, they were centrifuged to separate the char from the riboflavin solution, then a 100.0 μL aliquot was taken from each sample using a 100.0 μL micro-pipette and transferred to a 1.50 ml micro-centrifuge tip, then diluted to 1000 μL with ultrapure H_2O. This dilution was performed as the stock concentration used for adsorption is simply too concentrated and the microplate reader will produce the maximum possible signal, even after some adsorption takes place, which makes the data meaningless. So, if no adsorption were to occur at all, 51.8 mg/L after a $1/10^{th}$ dilution would be 5.18 mg/L which fits within the calibration curve.

Riboflavin Standard Curve(s): Riboflavin standards were created in triplicate to run on the microplate along with the samples to be analyzed. This allows for the construction of a calibration curve relating the fluorescence intensity of riboflavin to the concentration of riboflavin in solution. Each time an experiment's respective solutions were to be analyzed, these standards were in the plate at the same time as the solutions, as well as a solvent blank (ultrapure H_2O) in triplicate and a reagent blank consisting of the respective Riboflavin stock used for adsorption. This is done to ensure the environment and instrumental conditions (signal gain) are the same for both the standard solutions and "raw" samples.

To make these standards for the calibration curve, 0.0217 g (21.7 mg) of riboflavin was weighed in a tared-out 1.5 ml micro-centrifuge tube, using an analytical balance. To this aliquot, 1000 μL of ultrapure H_2O was added and then this tip was capped and vortexed to fully

dissolve the riboflavin. This solution (21.7 mg/ml, Riboflavin) was then subject to a ½ dilution, i.e., 500.0 μL was transferred to a new tip and then this was then diluted to 1000 μL with ultrapure H_2O, and this subsequently, was capped and vortexed. Another dilution was performed on this solution (10.9 mg/ml); 50.0 μL was transferred to a new tip, and this was again diluted to 1000 μL. Lastly, three aliquots of 10.0 μL of this solution (0.543 mg/ml) were transferred to 3 separate tips, and this was diluted to 1000 μL, which makes a 0.00543 mg/ml or 5.43 mg/L riboflavin solution. These dilutions were performed in this manner to ensure that the highest concentration on the calibration curve was higher than that of the solution used in adsorption. Using these three 5.43 mg/L riboflavin solutions, 7 more serial dilutions with a dilution factor of 1/2 were performed and these eight solutions in triplicate were the standards used for the calibration curve. The excitation wavelength range of riboflavin is reported in the literature to be from 200.0 nm to 550.0 nm, and the emission wavelength range is from 450.0 nm to 650.0 nm. The optical filters for the BMG Labtech© microplate reader used were for the ranges of 485-412nm for excitation, and 590-510nm for emission.

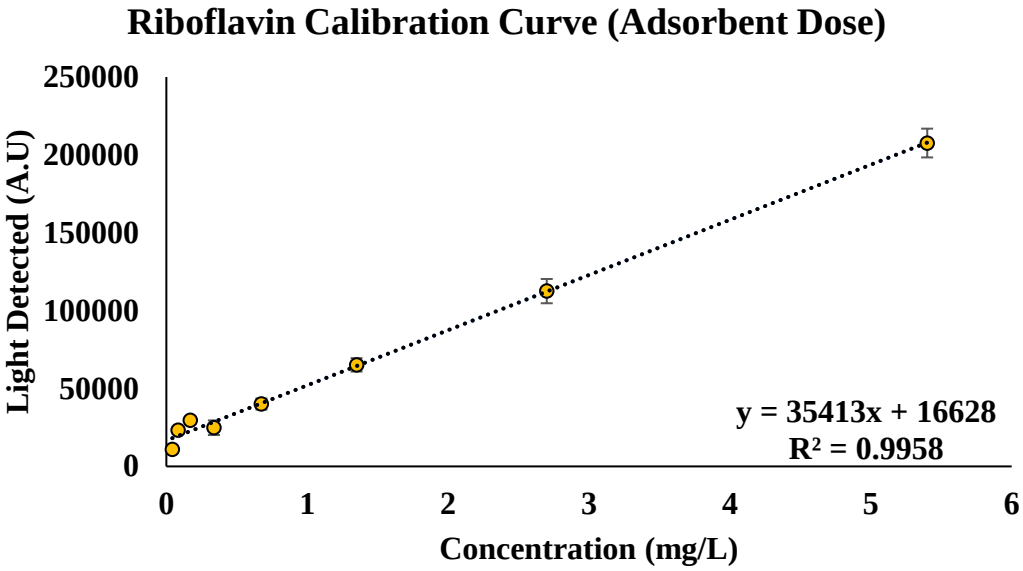


Figure 7: Riboflavin Calibration Curve for Adsorbent Dosage Experiment

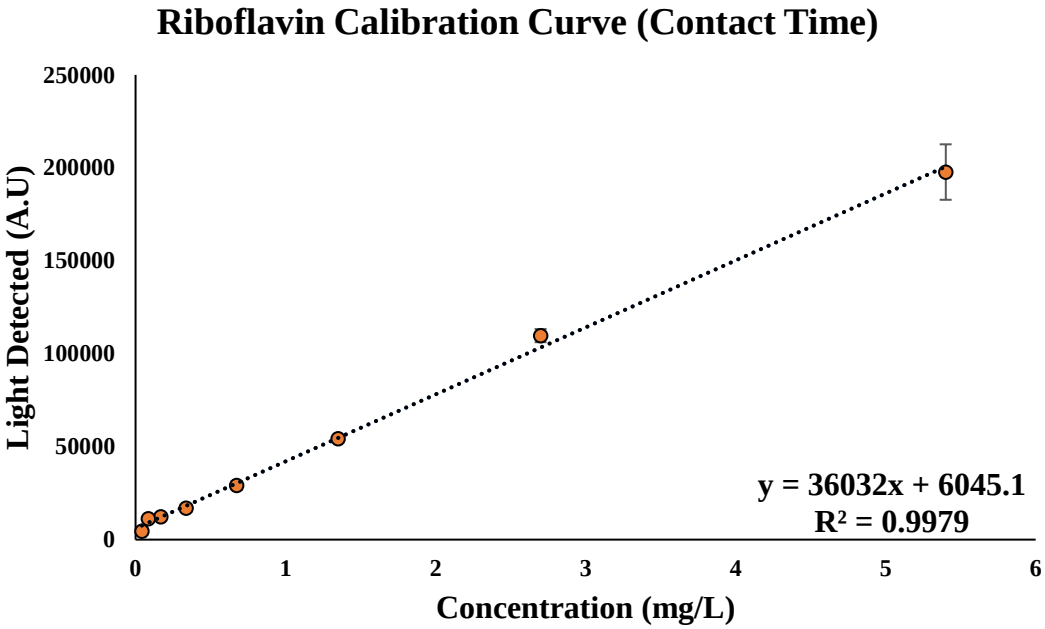


Figure 8: Riboflavin Calibration Curve for Contact Time (Kinetics) Experiment

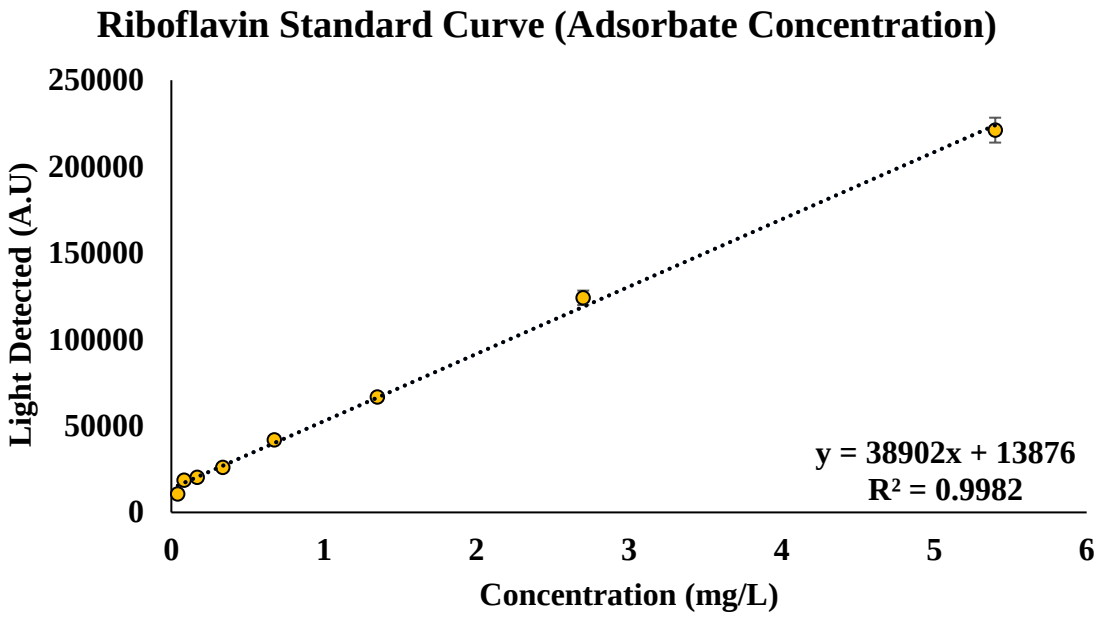


Figure 9: Riboflavin Calibration Curve for Adsorbate Concentration (Isotherm) Experiment

Contact Time Experiment: Investigating Adsorption Kinetics. This involved taking the optimum adsorbent dose, determined from the previous experiment, and then investigating different periods of time in which char is in contact with riboflavin in solution. This experiment, consisting again of triplicate measurement along with a method blank, involved keeping the char amount and riboflavin concentration constant, but checking the solutions after varying lengths of time. The periods of contact time investigated were: 120, 180, 240, 1440, and 2880 minutes. The procedure for this is the exact same as with adsorbent dose, except that 400.0 mg of char was used for the adsorbent dose.

Working Solution Concentration: Langmuir Isotherm Construction. An experiment was done to vary the initial concentration of the adsorbate (riboflavin) to construct a Langmuir Isotherm. The procedure for this was again, the same as the adsorbent dosage experiment, except for the fact that the char amount was kept constant, and the riboflavin solution concentration was varied.

Three new solutions were made by diluting the original 51.8 mg/L stock, to 40.0, 20.0, and 10.0 mg/L, respectively.

3. Results and Discussion

3.1 Characterization

3.1.1 Char Washing

For Soxhlet washing, initially the wash solution appears a deep-red colour. Each subsequent day of washing showed dilute to no color, until the fifth day when the wash was completely colorless. Wash 1 had an absorbance of 1.700 at a lambda max of ~290 nm and wash 5 had an absorbance of just over 0.100. This, along with the increase in surface area described above, shows a decrease in the number of ultraviolet-absorbing volatiles being purged from the char during washing.

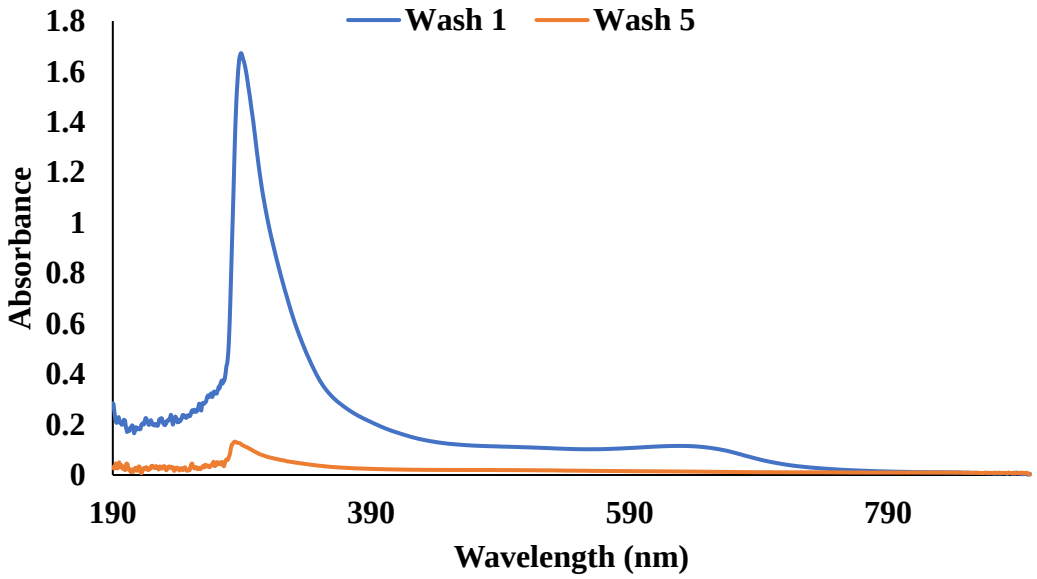


Figure 10: UV-Visible Spectrum of AcetaChar Washes

3.1.2 N_2 Physisorption

Nitrogen physisorption analysis was performed using a Micromeritics ASAP 2020 Sorptometer. This was performed for a sample of pristine Acetaminophen char after pyrolysis, as well as for a sample of this char after it was washed for five, 4 to 6-hour washes in a Soxhlet apparatus using a solvent mixture of 60:40 ultrapure water and 95% ethanol. The results are tabulated below and compared to literature biochar of similar origin. See Figures 11 and 12 for the N_2 adsorption-desorption isotherm of both the pristine and washed char, respectively.

From these results, we can see that compared to biochars produced from the sludge of many combined pharmaceuticals, the AcetaChar surface area is quite low. Surface area is not the only factor that influences adsorption, surface functionality also plays a role in this phenomenon, typically via hydrogen bonding, some ionic interactions, as well as $\pi - \pi$ stacking.

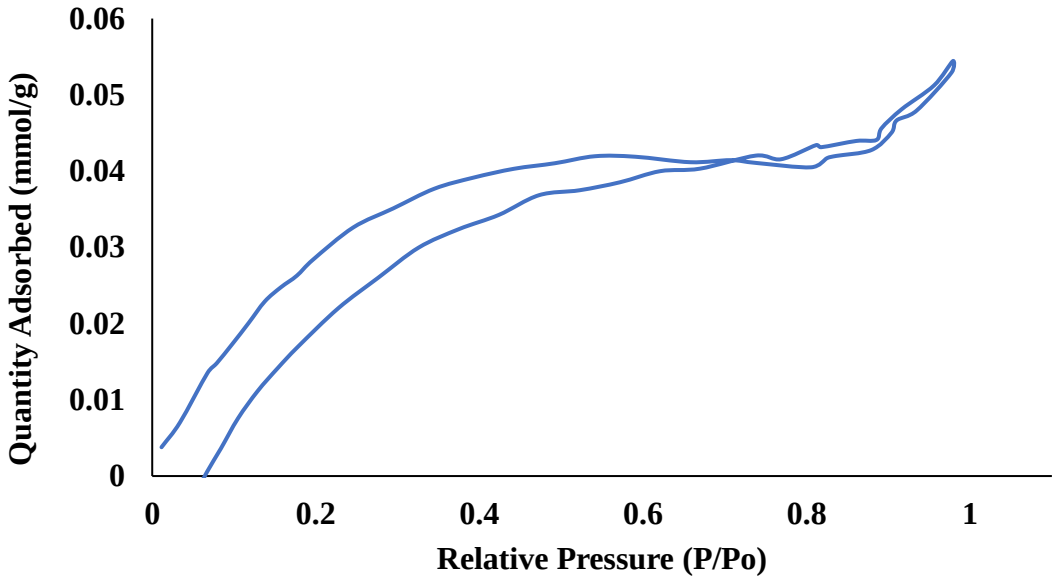


Figure 11: N2 Physisorption Isotherm (77K) Plot for Pristine AcetaChar

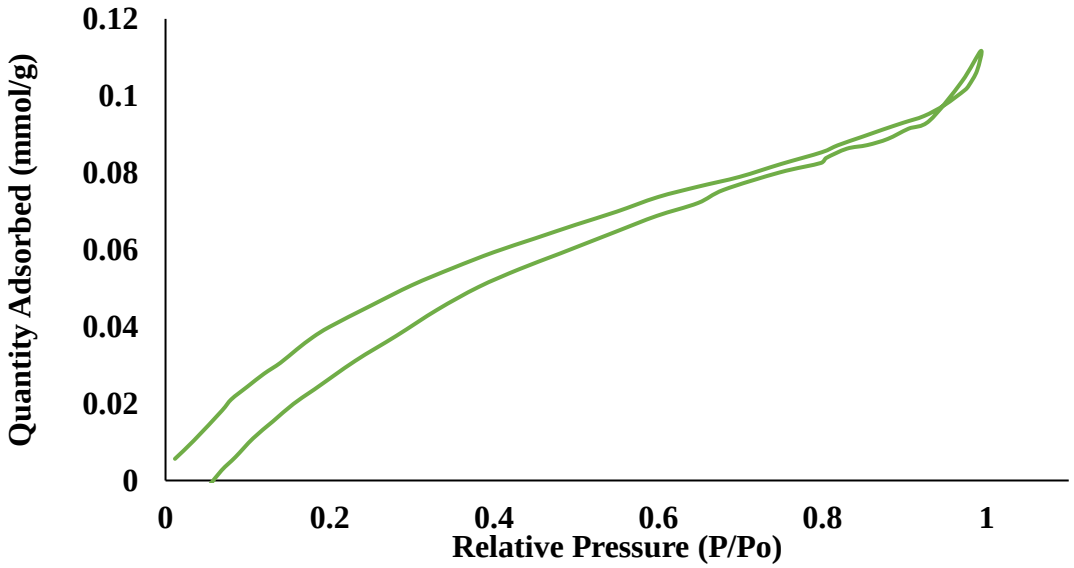


Figure 12: N2 Physisorption Isotherm (77K) Plot for Washed AcetaChar

Table 1: Pyrolysis Conditions for different PharmaChar and Associated B.E.T Surface Area Measurements

Char	Pyrolysis Conditions	B.E.T Surface Area	Reference
Pristine AcetaChar	50 g, 4.5°C/min to 800°C for 30 min	3.1592 m^2/g	-
Washed AcetaChar	-	4.5487 m^2/g	-
Pharma-Sludge Char (PBC800)	50 g, 10°C/min to 800°C for 90 min	264.05 m^2/g	(Wu et al., 2021)

3.1.3 TEM

TEM images were obtained for the washed AcetaChar, before and after the adsorption of riboflavin. TEM imaging was used to check multiple pieces of char and examine any morphological differences between the before and after images. TEM image of chars prior to adsorption (Figure 13) is consistent with thin sheets of amorphous material. In contrast, char images after

adsorption (Figure 14 and 15) show distinct, uniform, clusters across the surface which is consistent with the literature, with Riboflavin particles as a mixture of integrated and single particle forms adsorbed to the surface of the char[29].

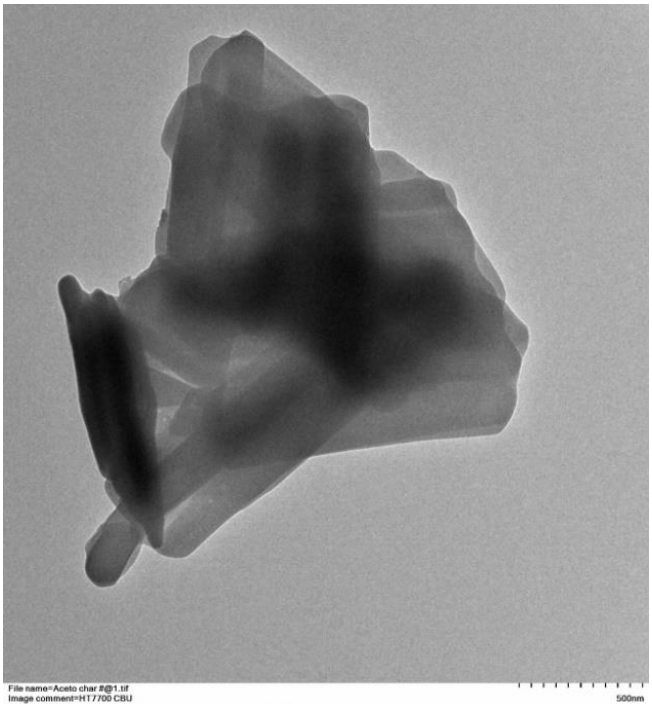


Figure 13: TEM Imaging of Char Before Adsorption

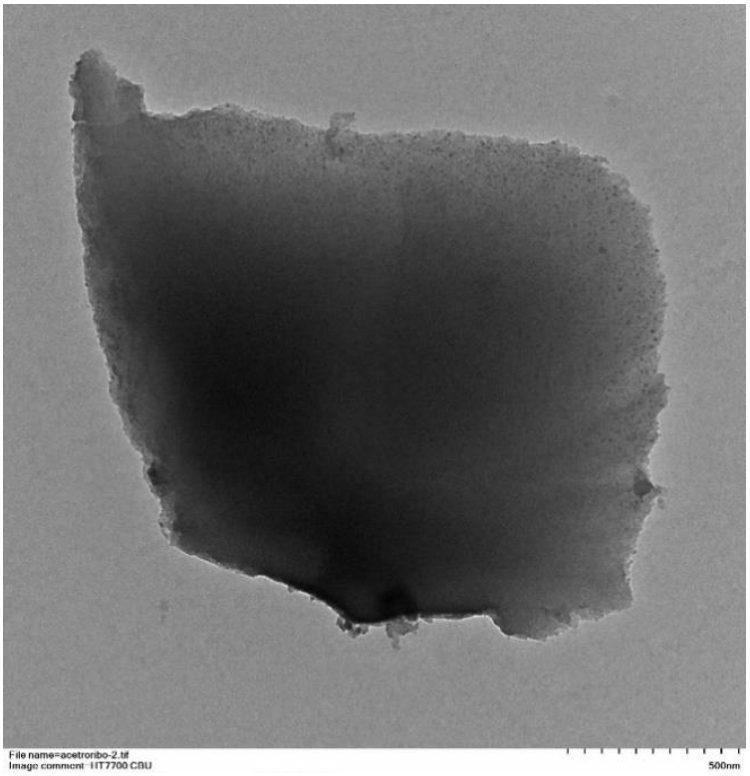


Figure 14: TEM Imaging of Char After Adsorption

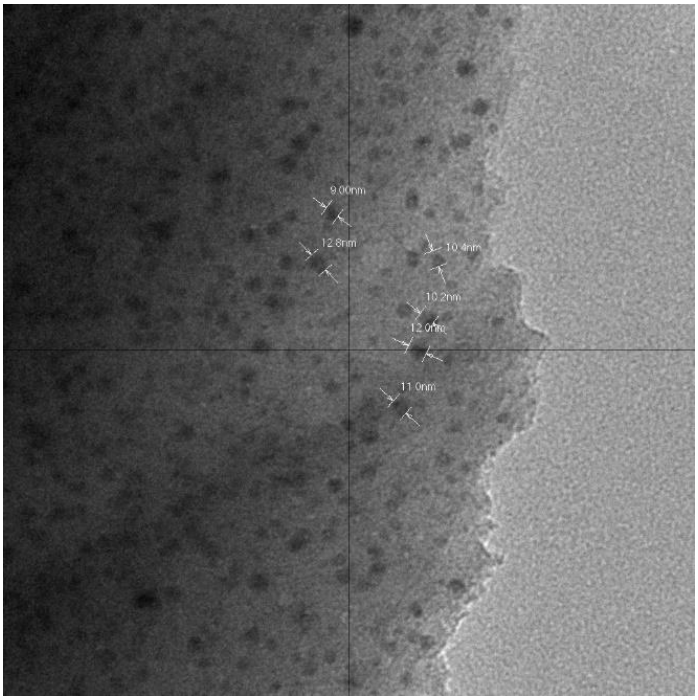


Figure 15: Figure 14, Magnified

3.1.4 Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR transmittance spectra are shown in Figures 17 and 18. In Figure 17, there is a clear retention of functional groups in the AcetaChar which are from the non-pyrolyzed aceta-minophen powder. In Figure 18, the appearance of key riboflavin functional groups in the Ribo-flavin-AcetaChar spectrum proves that adsorption was indeed successful.

Table 2: FTIR Peak Assignments for AcetaChar

Wavenumber (cm^{-1})	Functional Group
~3100	O-H Stretch
~2900	Alkyl C-H
~1650	C=O Amide
~1440	C=C
1000	C-O

Table 3: FTIR Peak Assignments for Riboflavin

Wavenumber (cm^{-1})	Functional Group
3491	N-H Stretch
3320	O-H Stretch
~3200-2800	Alkyl and Aromatic C-H
1731	C=O, Amide
1647	C=O, Amide
1553	N-H Bend
1540	C=C
1445	C-C
1384	C-N

Table 4: FTIR Peak Assignments for Riboflavin-AcetaChar

Wavenumber (cm^{-1})	Functional Group
3427	O-H (Possibly N-H as well)
~2850-2900	Alkyl and Aromatic C-H
1449	C-C
1384	C-N

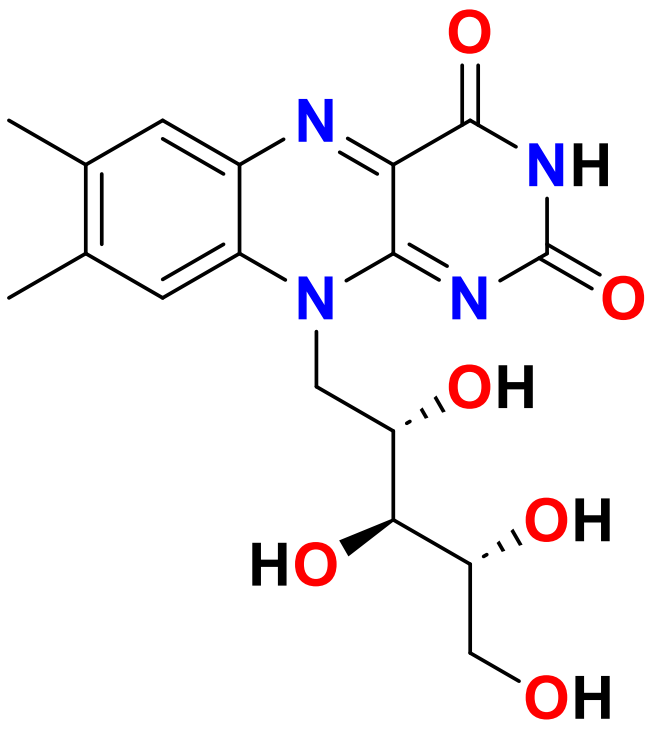


Figure 16: Riboflavin Molecule

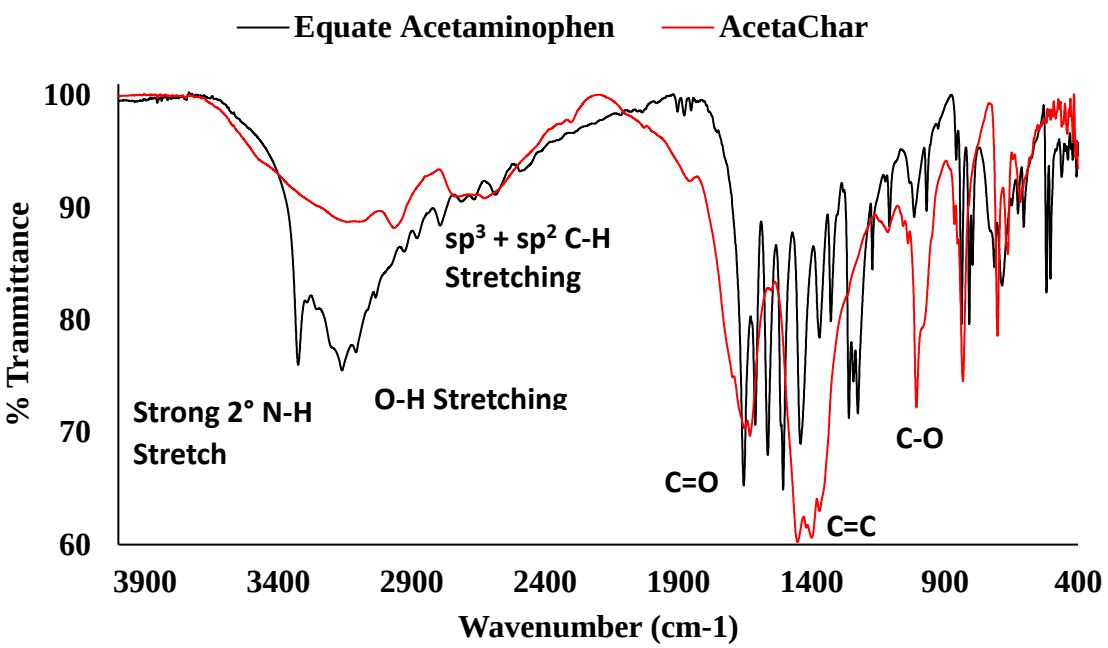


Figure 17: Infrared Spectra of Equate Acetaminophen and Char

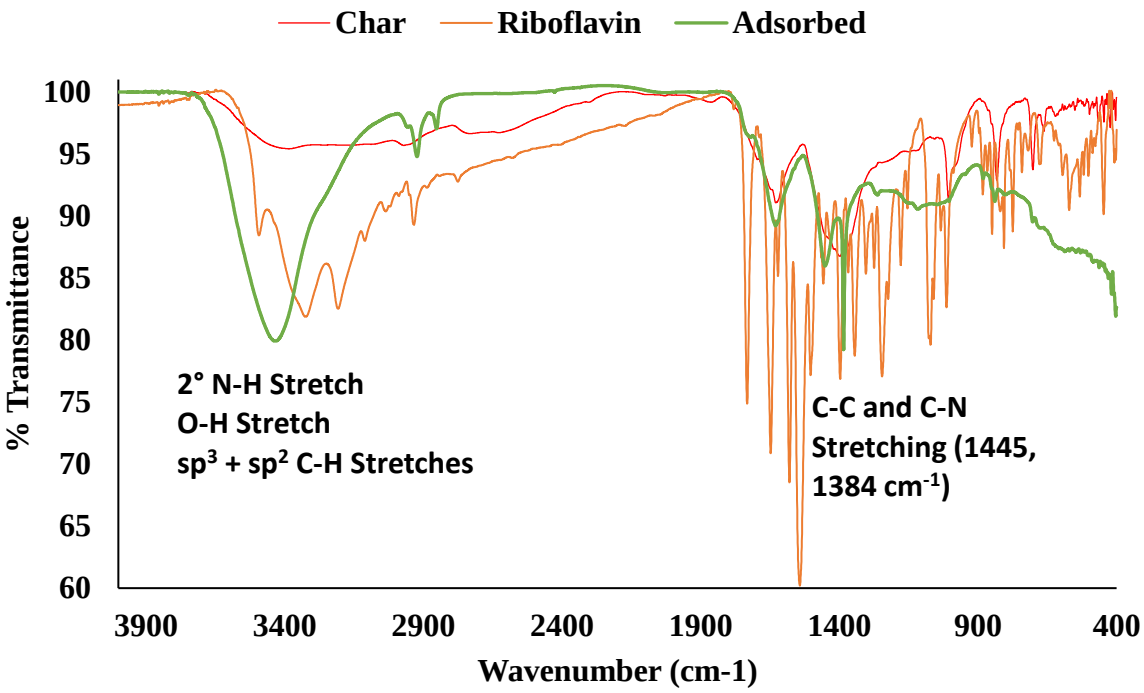


Figure 18: Infrared Spectra of Char, Riboflavin, and Riboflavin Adsorbed Char

3.1.5 Elemental Analysis

Table 5: Elemental Analysis Data for Acetaminophen-based PharmaChar

Element Present:	Carbon	Hydrogen	Nitrogen	Oxygen
Acetaminophen	63.56 %	6.00 %	9.27 %	21.17 %
AcetaChar	74.10 %	3.44 %	9.43 %	13.03 %

Theoretical elemental analysis can be calculated using the molecular formula of Acetaminophen ($C_8H_9NO_2$) and its molecular weight, 151.15 g/mol. Without accounting for the filler materials in the Acetaminophen tablets used, some deductions can be made. An increased carbon content and reduced hydrogen content is consistent with the pyrolysis process. Carbon condenses into aromatic ring systems and so hydrogen is lost due to de-saturation of C=C bonds. The loss of oxygen and hydrogen is also likely due to significant reduction in water content. Interestingly, nitrogen content is not only retained, but slightly increased as well. This is of considerable interest to future work as nitrogen functionality could contribute to the adsorption process.

3.2 Batch Adsorption Study

For each adsorption experiment, it was necessary for a calibration curve to be constructed for that set of samples. This is because when the BMG Labtech Microplate Reader takes a measurement, it scans each well of the microplate for the one which is producing the largest signal. Once this is determined, the instrument then adjusts the gain of the signal, which subsequently amplifies every other signal according to the largest. This is done every time a plate is put into the instrument and so with each set of samples, the gain will be different, and this will affect the readings slightly from run to run.

To determine the actual concentration of the working solution after adsorption, the calibration curve is used to calculate the concentration of the diluted working solution that is run on the instrument. This concentration is multiplied by the dilution factor used to dilute from the working solution to the microplate. This dilution involved taking 100.0 μL of the working solution, diluting to 1000 μL ($1/10^{th}$ dilution), and then running 200.0 μL of this in the microplate. Each concentration that is determined using a calibration curve must be multiplied by the inverse of the dilution factor, which is 10.

The calibration range of 0.042 – 5.43 mg/L was chosen as more concentrated solutions result in a maximum fluorescence intensity of 260,000 A.U. This is the upper limit for this plate reader, because of this each solution had to be diluted by a factor of 10 before being transferred to the micro-plate for analysis. Each calibration curve had an R^2 value of 0.99, which shows the micro-plate reader produces a signal that is linearly related and therefore, directly proportional, to the concentration of Riboflavin in solution.

3.2.1 Adsorbent Dose

Adsorbent dose optimization experiments shown in Figure 19 indicated that a 100 mg of char adsorbed on average 3.8% of a 51.8 mg/L riboflavin solution, with little variability among the triplicate samples. Upon doubling the concentration an enhanced adsorption was noted however it was still quite low. At 400 mg of char 400 mg of char adsorbed an average of 36.6% of the 51.8 mg/L solution of riboflavin. This experiment shows that increasing the amount of char does not show a completely linear increase in the amount of riboflavin adsorbed. The heterogenous nature of the AcetaChar is likely what causes such differences in % adsorbed, but this could also be due to experimental error.

Percent adsorbed was calculated using the following equation:

$$\frac{(Ci - Ce)}{Ce} * 100\%$$

Where C_i is the initial riboflavin concentration of the working solution and C_e is the riboflavin concentration of the working solution after adsorption has taken place.

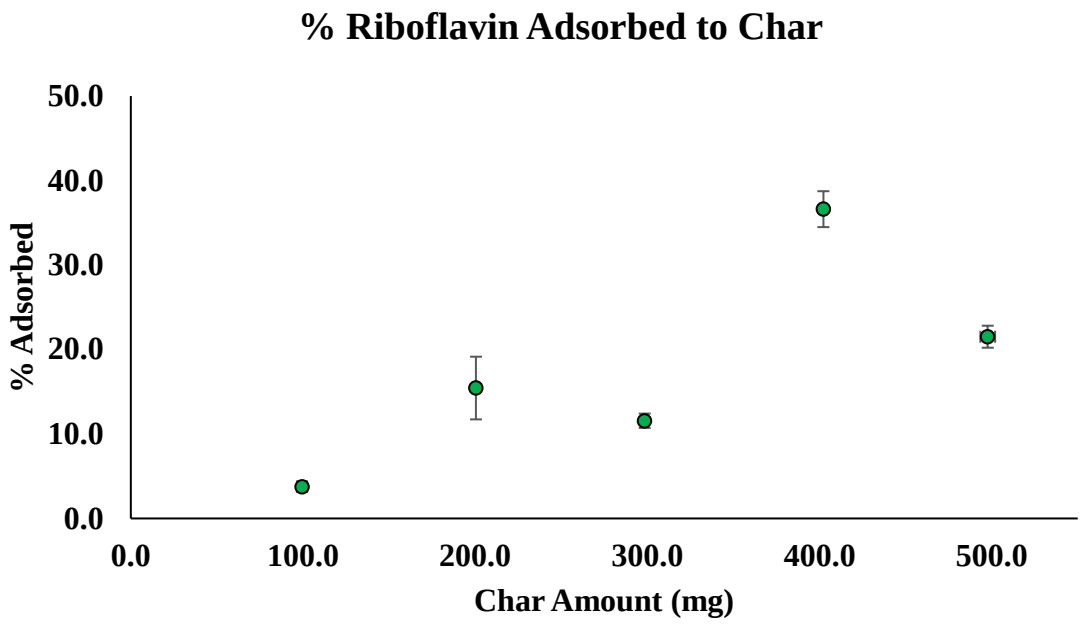


Figure 19: Average Percent of Riboflavin adsorbed for varying amounts of AcetaChar

3.2.2 Contact Time and Kinetics Study

Multiple contact times were investigated. These being: 2 hours, 3 hours, 4 hours, 24 hours, and 48 hours. For these experiments, char amount was kept constant at 400.0 mg. This amount was chosen based on the adsorbent amount experiment. The riboflavin solution used had a concentration of 51.8 mg/L. Overall, there was not much of an increase in adsorption beyond the 2-hour data points as seen in Figure 20 and Table 7. Given this result, it would be beneficial to look at much lower contact times in the range of 0 – 2 hours in 15-minute increments.

With this data, a simple Kinetics plot can be constructed to relate the amount adsorbed in milligrams or adsorbate per grams of adsorbent. Kinetics of adsorption is of considerable interest when determining the possible applications of char materials. This plot does not show a linear increase of amount adsorbed with increasing contact time and while this may be due to the heterogenous nature of char material, it is entirely likely the process has reached the point at which the maximum amount of riboflavin has adsorbed on the surface of the char. As Wang et. al. has shown, adsorption is likely to occur quite rapidly[24]. This reiterates the need to investigate shorter contact times and possibly more dilute adsorbate concentrations.

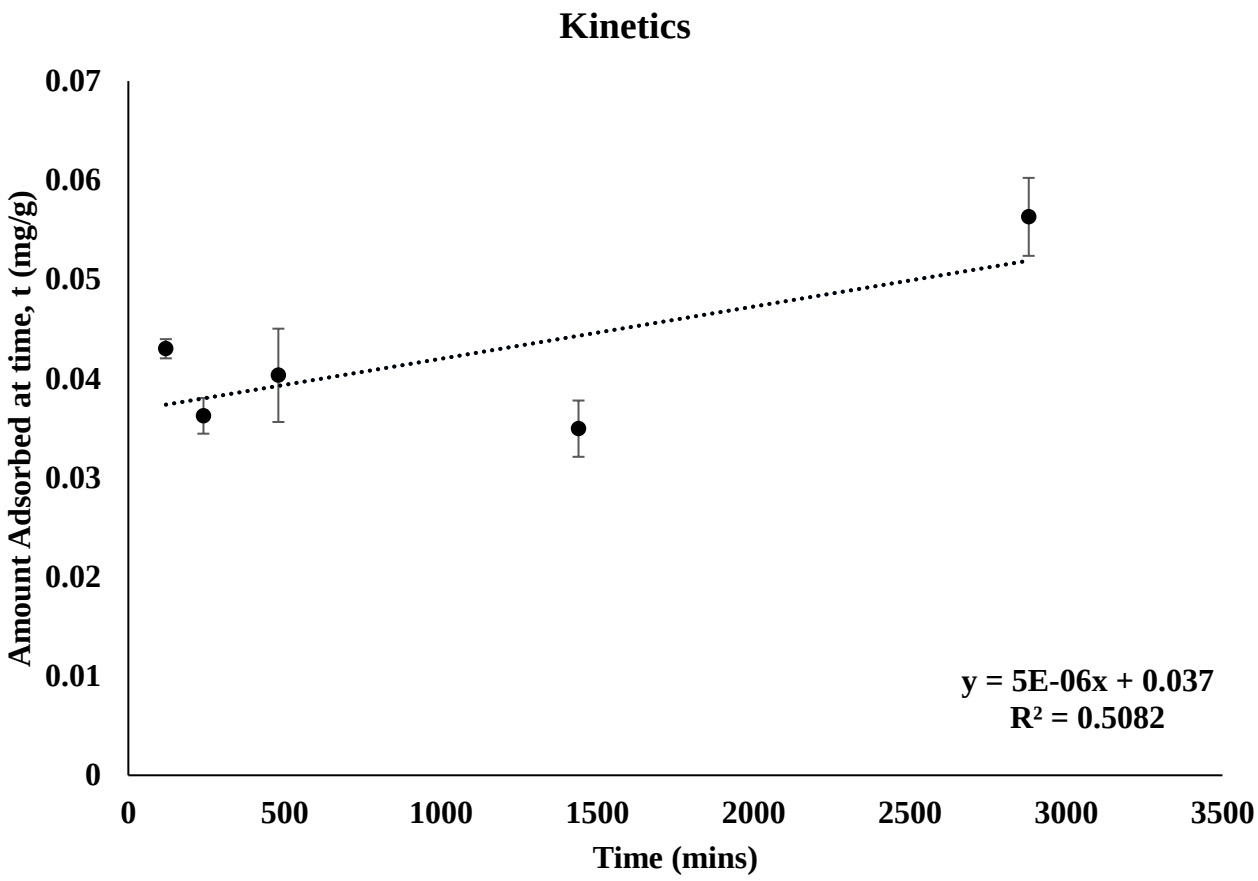


Figure 20: Kinetics Plot of Amount Adsorbed vs. Contact Time

3.2.3 Adsorbate Concentration and Isotherm Study

Isotherms are a valuable tool for describing adsorption, some of the interactions between adsorbate and adsorbent, and adsorbent-adsorbate interactions. A Langmuir Isotherm is a typical isotherm used in novel adsorption studies that assumes adsorption sites are far enough apart to have little-to-no adsorbate-adsorbate interactions. Meaning adsorption at one site should not affect another. The Langmuir model also assumes that saturation occurs with monolayer coverage and that pores do not contribute to the sorption mechanism[30,31]. To construct this isotherm, the initial adsorbate concentration (C_i) is varied, and the equilibrium adsorbate concentration corresponding to each C_i is then plotted together as Ce vs. Ci. It is imperative to mention that this isotherm is not typically what is described in the literature, this method was used to gain some understanding of what is occurring with the adsorbate concentrations that were investigated. The typical plot for Langmuir isotherm fitting-models would be a plot of C_e/q_e vs. C_e, however due to reaching the saturation point, not much can be gleamed from this plot, and it was considered inconclusive, see Figure 17 in Appendix 2.

From the curve in Figure 25, a similar result to the kinetics plot can be seen, in that, it seems the process reached the saturation point and the most amount of riboflavin adsorbed is roughly 0.02 mg of riboflavin per gram of AcetaChar. While this amount initially seems quite low, given the low surface area calculated prior, this is expected. Along with that, the goal of this project is to use the Char as a support for riboflavin to be used as an organo-catalyst, and so only a small amount is required to be present for catalytic activity.

With this information, in the future it would be of interest to either dilute the adsorbate solutions significantly, or increase the amount of char used, and to subsequently use this data to construct a new Langmuir Isotherm as well as others to determine the degree of surface

heterogeneity that the char displays and whether the mechanism of adsorption is more physisorption or chemisorption.

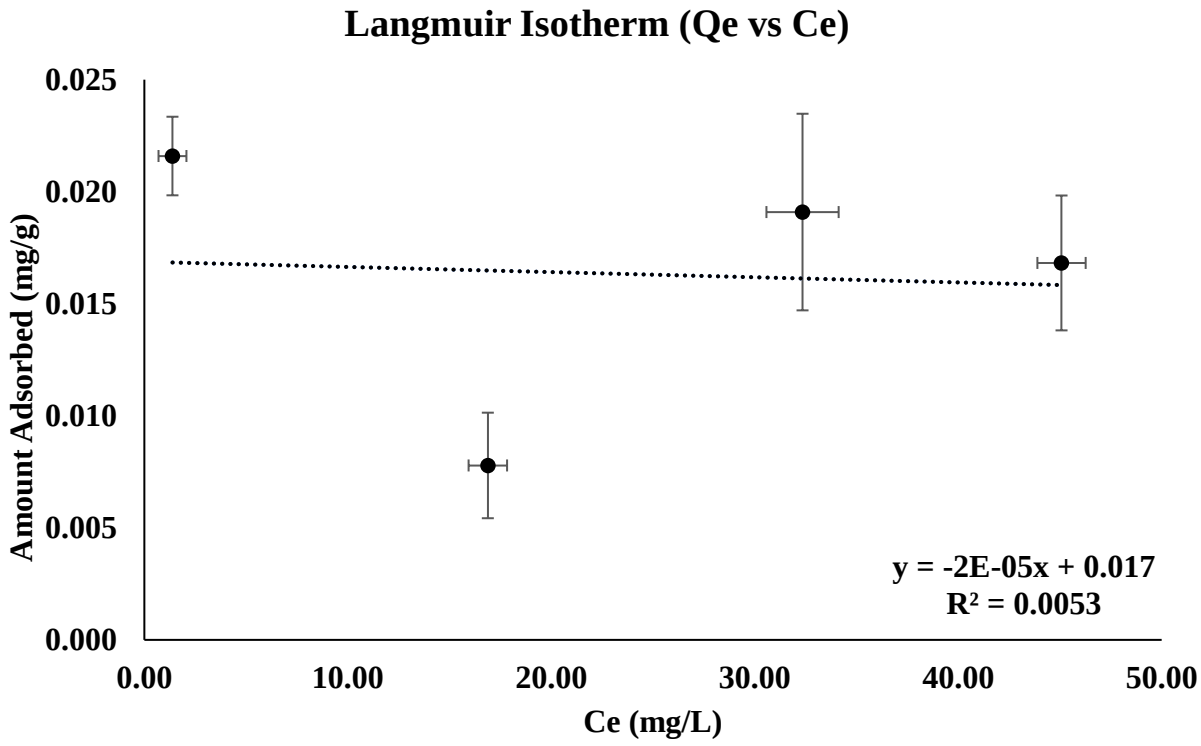


Figure 21: Simple Langmuir Isotherm

4. Conclusions and Future Work

This study investigated the use of AcetaChar as an adsorbent for removing riboflavin from aqueous solutions, with the goal of creating a renewable and heterogenous support for riboflavin as a photo-redox catalyst. The results showed that Soxhlet washing resulted in a decrease in ultraviolet-absorbing volatiles being purged from the char during washing with a water:ethanol solvent mixture, resulting in an increased surface area. With that, it may be of interest to try other solvents and/or mixtures of solvents to expose more, or different active sites, in the future.

Compared to PharmaChars produced from the sludge of many combined pharmaceuticals, the AcetaChar surface area is still quite low. Given this result, it may be of interest to either perform physical/chemical activation on AcetaChar or, combine acetaminophen and other expired pharmaceuticals for pyrolysis to see if this either provides larger surface area, or increased functionality. TEM images revealed uniform clusters of riboflavin particles adsorbed onto the surface of the char. With that, the FTIR spectra showed the retention of functional groups in the char from the non-pyrolyzed acetaminophen powder along with the appearance of key riboflavin functional groups in the Riboflavin-Char spectrum, proving that adsorption was indeed successful. The elemental analysis showed an increased carbon content and reduced hydrogen content, consistent with the pyrolysis process. Overall, the study suggests that AcetaChar has potential as an adsorbent for removing riboflavin from aqueous solutions, further research is required to optimize the process.

5. Works Cited

(1) *Canadian Pharmaceutical Trends*. https://www.iqvia.com/locations/canada/canadian-pharmaceutical-trends (accessed 2022-09-19).

(2) Tong, A.; Peake, B.; Braund, R. Disposal Practices for Unused Medications in New Zealand Community Pharmacies. *J. Prim. Health Care* **2011**, *3* (3), 197. https://doi.org/10.1071/HC11197.

(3) *Drugs in the water*. Harvard Health. https://www.health.harvard.edu/newsletter_article/drugs-in-the-water (accessed 2022-09-19).

(4) Lindberg, R. H.; Östman, M.; Olofsson, U.; Grabic, R.; Fick, J. Occurrence and Behaviour of 105 Active Pharmaceutical Ingredients in Sewage Waters of a Municipal Sewer Collection System. *Water Res.* **2014**, *58*, 221–229. https://doi.org/10.1016/j.watres.2014.03.076.

(5) *Health-care waste*. https://www.who.int/news-room/fact-sheets/detail/health-care-waste (accessed 2023-03-27).

(6) Kadam, A.; Patil, S.; Patil, S.; Tumkur, A. Pharmaceutical Waste Management An Overview. *INDIAN J. Pharm. Pract.* **2016**, *9*, 2–8.

(7) *Medical waste management - A review | Elsevier Enhanced Reader*. https://doi.org/10.1016/j.jenvman.2015.08.013.

(8) Mondal, S.; Bobde, K.; Aikat, K.; Halder, G. Biosorptive Uptake of Ibuprofen by Steam Activated Biochar Derived from Mung Bean Husk: Equilibrium, Kinetics, Thermodynamics, Modeling and Eco-Toxicological Studies. *J. Environ. Manage.* **2016**, *182*, 581–594. https://doi.org/10.1016/j.jenvman.2016.08.018.

(9) Liu, H.; Xu, G.; Li, G. The Characteristics of Pharmaceutical Sludge-Derived Biochar and Its Application for the Adsorption of Tetracycline. *Sci. Total Environ.* **2020**, *747*, 141492. https://doi.org/10.1016/j.scitotenv.2020.141492.

(10) Pyrolyzing Pharmaceutical Sludge to Biochar as an Efficient Adsorbent for Deep Removal of Fluoroquinolone Antibiotics from Pharmaceutical Wastewater: Performance and Mechanism. *J. Hazard. Mater.* **2022**, *426*, 127798. https://doi.org/10.1016/j.jhazmat.2021.127798.

(11) *Concentration of Atmospheric Pollutants in the Gaseous Emissions of Medical Waste Incinerators*. https://www.tandfonline.com/doi/epdf/10.1080/10473289.2000.10463989?needAccess=true&role=button (accessed 2023-03-27).

(12) Yu, Z.; Yousaf, K.; Ahmad, M.; Yousaf, M.; Gao, Q.; Chen, K. Efficient Pyrolysis of Ginkgo Biloba Leaf Residue and Pharmaceutical Sludge (Mixture) with High Production of Clean Energy: Process Optimization by Particle Swarm Optimization and Gradient Boosting

Decision Tree Algorithm. *Bioresour. Technol.* **2020**, *304*, 123020. https://doi.org/10.1016/j.biortech.2020.123020.

(13) Tomul, F.; Arslan, Y.; Kabak, B.; Trak, D.; Kendüzler, E.; Lima, E. C.; Tran, H. N. Peanut Shells-Derived Biochars Prepared from Different Carbonization Processes: Comparison of Characterization and Mechanism of Naproxen Adsorption in Water. *Sci. Total Environ.* **2020**, *726*, 137828. https://doi.org/10.1016/j.scitotenv.2020.137828.

(14) Czech, B.; Kończak, M.; Rakowska, M.; Oleszczuk, P. Engineered Biochars from Organic Wastes for the Adsorption of Diclofenac, Naproxen and Triclosan from Water Systems. *J. Clean. Prod.* **2021**, *288*, 125686. https://doi.org/10.1016/j.jclepro.2020.125686.

(15) Laird, D. A.; Brown, R. C.; Amonette, J. E.; Lehmann, J. Review of the Pyrolysis Platform for Coproducing Bio-Oil and Biochar. *Biofuels Bioprod. Biorefining* **2009**, *3* (5), 547–562. https://doi.org/10.1002/bbb.169.

(16) Liang, C.; Sun, H.; Ling, C.; Liu, X.; Li, M.; Zhang, X.; Guo, F.; Zhang, X.; Shi, Y.; Cao, S.; He, H.; Ai, Z.; Zhang, L. Pyrolysis Temperature-Switchable Fe-N Sites in Pharmaceutical Sludge Biochar toward Peroxymonosulfate Activation for Efficient Pollutants Degradation. *Water Res.* **2023**, *228*, 119328. https://doi.org/10.1016/j.watres.2022.119328.

(17) Agrawal, S.; Khazaeni, B. Acetaminophen Toxicity. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2023.

(18) Acetaminophen in Drinking Water. **2014**.

(19) Lu, M.-C.; Chen, Y. Y.; Chiou, M.-R.; Chen, M. Y.; Fan, H.-J. Occurrence and Treatment Efficiency of Pharmaceuticals in Landfill Leachates. *Waste Manag.* **2016**, *55*, 257–264. https://doi.org/10.1016/j.wasman.2016.03.029.

(20) Pinto, J. T.; Zempleni, J. Riboflavin. *Adv. Nutr.* **2016**, *7* (5), 973–975. https://doi.org/10.3945/an.116.012716.

(21) Balasubramaniam, S.; Yaplito-Lee, J. Riboflavin Metabolism: Role in Mitochondrial Function. *J. Transl. Genet. Genomics* **2020**. https://doi.org/10.20517/jtgg.2020.34.

(22) Morack, T.; Metternich, J. B.; Gilmour, R. Vitamin Catalysis: Direct, Photocatalytic Synthesis of Benzocoumarins via (−)-Riboflavin-Mediated Electron Transfer. *Org. Lett.* **2018**, *20* (5), 1316–1319. https://doi.org/10.1021/acs.orglett.8b00052.

(23) Ma, X.; Chen, Z.; Sun, Y.; Cai, Z.; Cheng, F.; Ma, W. Effect on Kinetics and Energy Distribution of Riboflavin Adsorption from Magnetic Nano-Carbon Composites with Adsorbed Water Layer. *Sep. Purif. Technol.* **2022**, *292*, 120995. https://doi.org/10.1016/j.seppur.2022.120995.

(24) Wang, Y.-S.; Zhi, W.-R.; Jiang, H.; Zhao, Y.-H.; Li, Z.-X.; Luo, S.-Q.; Zhang, S.-Q.; Huang, P.-P.; Wang, L.-F.; Liu, B. Unraveling the Mechanism of Efficient Adsorption of Riboflavin onto Activated Biochar Derived from Algal Blooms. *J. Environ. Manage.* **2021**, *291*, 112725. https://doi.org/10.1016/j.jenvman.2021.112725.

(25) Comparing Physisorption and Chemisorption Solid Sorbents for Use Separating CO2 from Flue Gas Using Temperature Swing Adsorption. *Energy Procedia* **2011**, *4*, 562–567. https://doi.org/10.1016/j.egypro.2011.01.089.

(26) *Equate Extra Strength Acetaminophen Caplets, 500 mg, 500 Ct*. Walmart.com. https://www.walmart.com/ip/Equate-Extra-Strength-Acetaminophen-Caplets-500-mg-500-Ct/39661524 (accessed 2022-08-22).

(27) Dunnigan, L.; Morton, B. J.; Ashman, P. J.; Zhang, X.; Kwong, C. W. Emission Characteristics of a Pyrolysis-Combustion System for the Co-Production of Biochar and Bioenergy

from Agricultural Wastes. *Waste Manag.* **2018**, *77*, 59–66. https://doi.org/10.1016/j.wasman.2018.05.004.

(28) *The Transmission Electron Microscope | CCBER*. https://www.ccber.ucsb.edu/ucsb-natural-history-collections-botanical-plant-anatomy/transmission-electron-microscope (accessed 2023-04-01).

(29) Rezaei-Zarchi, S.; Javed, A.; Mirjalili, H.; Abarghouei, H. B.; Hashemizadeh, S. A. Characterization and Electrochemical Study of Nano-Composition Based Methylene Blue- and Riboflavin-Nafion on the Surface of Gold Electrode. *Turk. J. Chem.* **2009**. https://doi.org/10.3906/kim-0812-25.

(30) Abin-Bazaine, A.; Trujillo, A. C.; Olmos-Marquez, M. *Adsorption Isotherms: Enlightenment of the Phenomenon of Adsorption*; IntechOpen, 2022. https://doi.org/10.5772/intechopen.104260.

(31) Ayawei, N.; Ebelegi, A. N.; Wankasi, D. Modelling and Interpretation of Adsorption Isotherms. *J. Chem.* **2017**, *2017*, e3039817. https://doi.org/10.1155/2017/3039817.

6. Appendices

Appendix 1 – Additional TEM Data

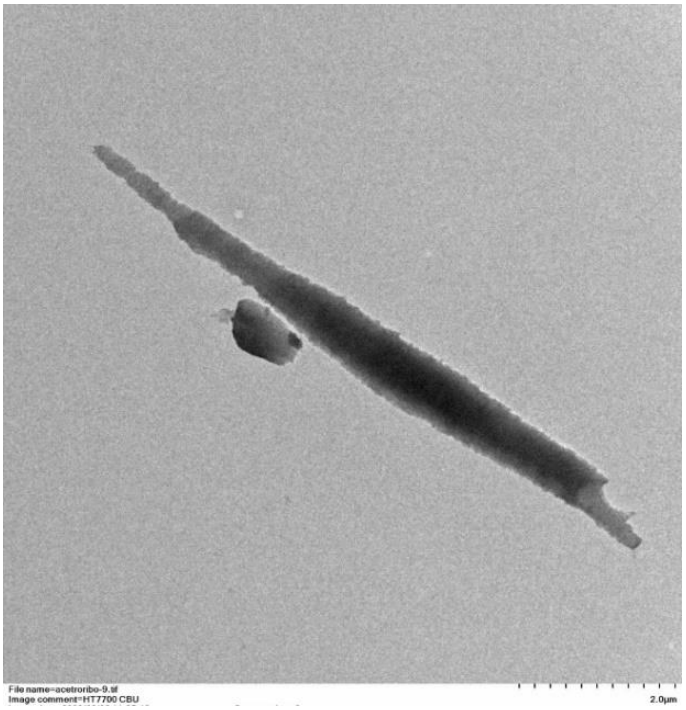


Figure 22: TEM Image After Adsorption

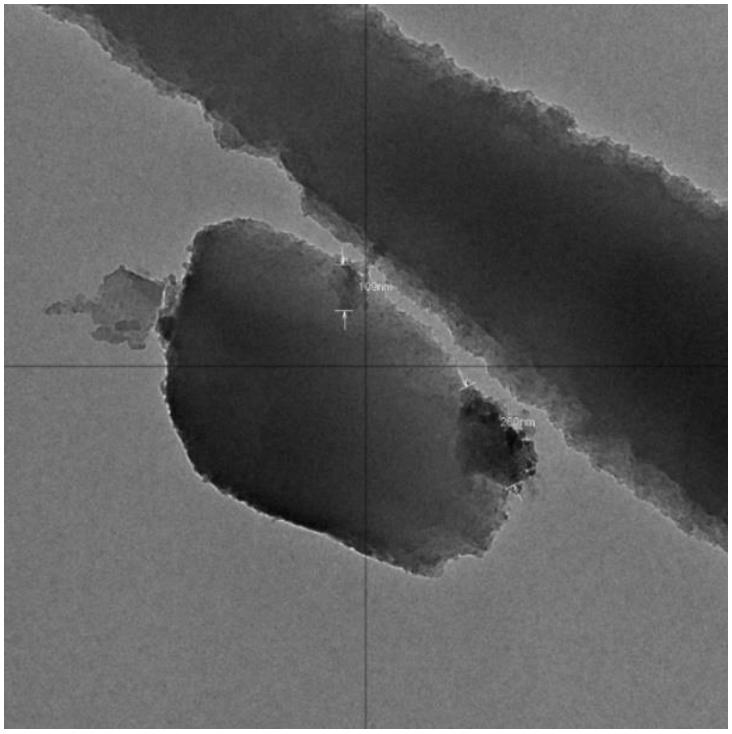


Figure 23: Figure 22 Magnified

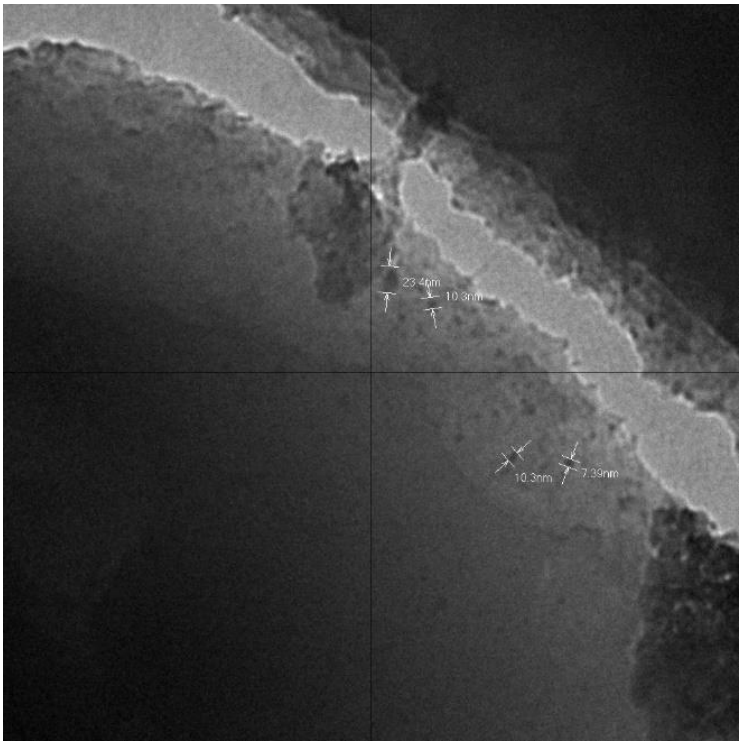


Figure 24: Figure 23 Magnified

Appendix 2 – Raw Data And Additional Figures

Table 6: % Sorption Raw Data

Char amount (g)	Light De-tected	Concentration of Working Solution, C_e (mg/L)	% Sorption
0.1013	192848	49.8	3.90
0.0991	190671	49.2	5.10
0.0980	195652	50.6	2.40
0.2005	188126	48.4	6.50
0.1974	161196	40.8	21.2
0.2036	164585	41.8	19.3
0.2978	176912	45.3	12.6
0.2965	182360	46.8	9.70
0.3011	176274	45.1	13.0
0.4019	139772	34.8	32.9
0.4027	132725	32.8	36.7
0.4022	123039	30.1	42.0
0.5037	155774	39.3	24.1
0.5022	165727	42.1	18.7
0.4873	158451	40.1	22.7

$y = 35413x + 16628$; $R^2 = 0.9958 \rightarrow \frac{192848 - 16628}{35413} = 4.976 \frac{mg}{l}\ (diluted) * 10 =$

$49.76 \frac{mg}{L} (in\ solution)$

Table 7: Contact Time Experiment Raw Data

Contact Time (mins)	Light Detected (A.U)	Adsorbate Concentration After Adsorption, C_e (mg/L)	Amount Adsorbed, q_e (mg/g)
120	128364	34.0	0.04463
120	129718	34.3	0.04369
120	134027	35.5	0.04070
240	146719	39.0	0.03190
240	136444	36.2	0.03903
240	138204	36.7	0.03780
480	134757	35.7	0.03618
480	139686	37.9	0.03310
480	109877	28.8	0.05171
1440	141652	37.6	0.02833
1440	127048	33.6	0.03644
1440	120475	31.8	0.04008
2880	117078	30.8	0.04722
2880	99635	26.0	0.05811
2880	90841	23.5	0.06360

$y = 36032x + 6045.1$; $R^2 = 0.9979$

Table 8: Adsorbate Concentration Raw Data

Char Amount (grams)	Initial Adsorbate Concentration, C_i (mg/L)	Light Detected (A.U)	Adsorbate Concentration After Adsorption, C_e (mg/L)	Amount Adsorbed, q_e (mg/g)
0.3999	51.8	190625	45.4	0.01592
0.4026	51.8	198260	47.4	0.01094
0.398	51.8	178835	42.4	0.02361
0.3958	40.0	156584	36.7	0.00838
0.4017	40.0	132406	30.5	0.02373
0.4014	40.0	130178	29.9	0.02517
0.4024	20.0	77607	16.4	0.00899
0.4001	20.0	72900	15.2	0.01207
0.3965	20.0	88139	19.1	0.00230
0.3999	10.0	25089	2.88	0.01780
0.4006	10.0	18887	1.29	0.02175

*Third trial of 10 mg/L was omitted as the signal was in the noise.

$y = 38902x + 13876$; $R^2 = 0.9982 \rightarrow \frac{190625 - 13876}{38902} = x = 4.543442497 * 10 = 45.4\ mg/L$

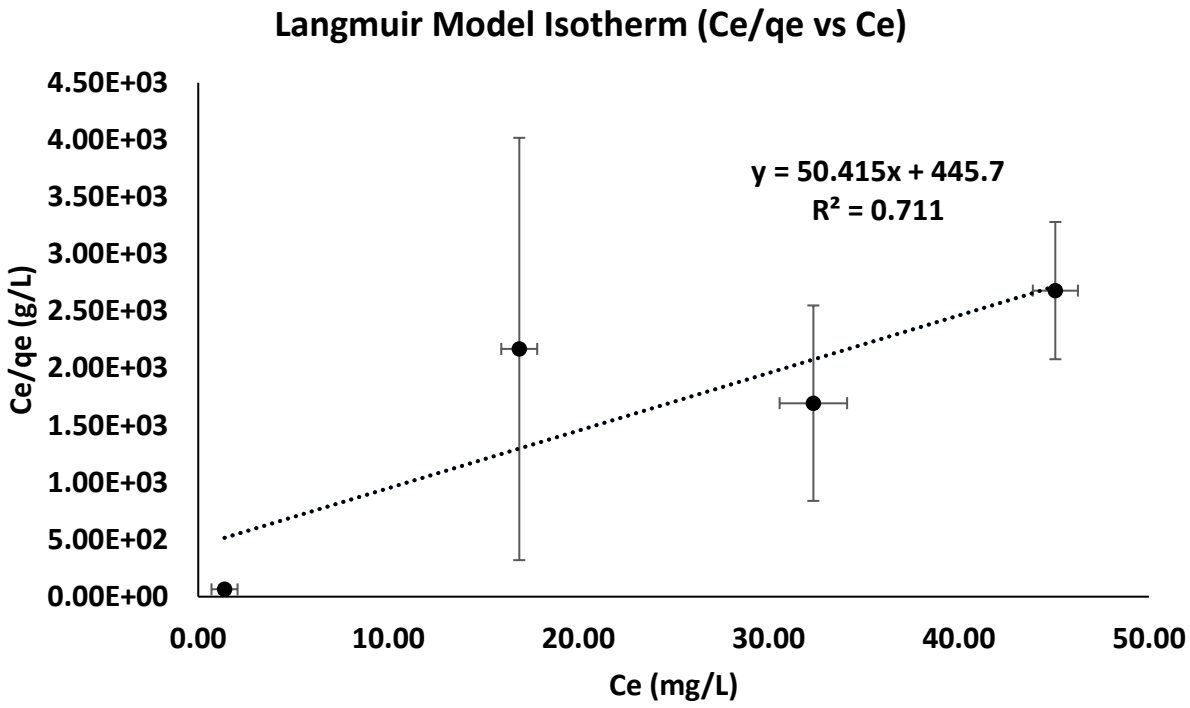


Figure 25: Traditional Langmuir Isotherm Model