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ASSESSING THE REACTIVITY OF AMINO ACIDS TO CHLORINATION USING CAPILLARY ELECTROPHORESIS

A Thesis

Presented to the

Faculty of

California State University,

San Bernardino

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Environmental Sciences

by

Pedro De Allende

August 2024

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ABSTRACT

Amino acids (AA) are ubiquitous in wastewater systems. One of the most popular wastewater treatments is chlorination which is effective at eradicating bacteria and overall treating the wastewater, but it can also produce disinfection by-products (DBPs). Certain DBPs have been classified as Class B Carcinogens to humans and are therefore highly regulated and monitored. Past studies have shown that natural organic matter (NOM), specifically amino acids, can be precursors to DBPs if chlorinated to the correct dose. This thesis project explored AA chlorination's mechanism(s) to understand further which products arise. In particular, the investigation detailed which steps of the mechanism vary depending on the amino acid and the chlorine dose, and which different DBPs are produced. Capillary electrophoresis (CE) will provide a different viewpoint. The hypothesis is that CE can detect DBP ions due to their detection based on electrophoretic mobility. Insight into the reactivity of individual amino acids undergoing chlorination will help understand their reactivity in mixtures and further guide the development of effective water treatment practices. From the experiments, what has been proven is that CE can track an AA chlorination reaction, and it can detect the DBP ion products. The experiments found the proposed DBP ions to have migration times between 4.73 and 8.55 minutes. Throughout the reaction, the product peak was shown to decrease in size for all AAs except for Glutamic Acid, Aspartic Acid, Lysine, and Proline during the 0.5:1 reactions. During the 1:1 reactions, the same is true but Serine also has a non-

reacting product peak. Based on the different products seen in each of the doses it can be surmised that each amino acid will react differently to the Cl_2 depending on what dose is injected. To further delineate the identity of the DBP ions, Mass Spectroscopy will be used in a future study.

ACKNOWLEDGEMENTS

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CHAPTER ONE

INTRODUCTION

Background and Context

Amino Acids and their Sources/Roles in Wastewater Treatment

Amino acids (AAs) are an integral part of organismal biochemistry. In a biological system, AAs are the building blocks of larger protein molecules. Proteins are paramount for organismal metabolic processes, as such they will find themselves in the wastewater from the native organisms and anthropogenic activities. Specifically upstream sewage, biological metabolism such as from algae, and animal husbandry sewage introduce a large concentration of AAs into wastewater (Cai et al., 2019). A past study found that AA concentration in natural waters ranges from 20 to 10,000 ug/L and combined amino acids are 4 to 5 more times abundant than free amino acids (Hong et al, 2008). Other than that, the AAs are most of the natural organic matter (NOM) in wastewater, and NOM is a large component of wastewater.

Significance of Studying Amino Acid Chlorination

AAs routinely interact with chlorine during the chlorination of wastewater. Chlorination is routinely used in the disinfection of wastewater, and it has been shown to prevent waterborne infectious diseases by removing harmful bacteria (Trehy et al., 1986). Chlorine is widely used for reasons such as price and retentive power (Brosillon, et al. 2009). Despite its ability to eradicate free bacteria, a major drawback is its production of toxic products such as DBPs

(Lazarova et al. 1999). In the water treatment stage, the concentration of amino acids is also liable to increase, paired with the fact that they have high reactivity with chlorine indicating a possible solution is to remove them (Hong et al., 2008). It is a necessity to remove them due to their production disinfection by-products (DBPs) (Hong et al., 2008). In a laboratory setting 600-700 DBPs have been observed to form from amino acid chlorination (Kanchanamayoon, 2015). Although only a few have been observed in reclaimed water the possibility of them forming is realistic. Examples of these DBPs include acetonitriles, acetaldehydes, trihalomethanes (THMs), hydrogen cyanide (HCN), halo acetic acids (HAA), and halo-acetonitriles (HAN) (Lazarova et al., 1999). None of these have been reported to be detected by capillary electrophoresis (CE) but it has the potential to detect them.

Capillary Electrophoresis as a Tool for Analysis.

Capillary electrophoresis (CE) would be a boon to current and future studies. CE works here because of the nature of our molecules of interest. Amino acids in a high pH buffer will be charged species, this charge is what will be necessary for the CE to separate the products of the chlorination reaction. While there are studies on the proposed reaction mechanisms, qualitatively analyzing the reactions with CE has largely been untouched (How et al., 2017). The hypothesis is that the reactivity of amino acids to chlorination will vary based on the chemical properties of the AA, the molar ratio of chlorine introduced to the amount of AA present, and the pH of the reaction mixture. Our main question

with these studies is what type of DBP ions can CE detect? CE will be the tool used to characterize their formation and decomposition as a function of AA type and chlorine to AA molar ratio.

Scope and Limitations of the Study

Delimitations of the Study

This project's scope entails all 20 AA ions. Previous papers operate under the assumption that all AAs will react similarly. The neutral species may be observed in a zone with the electroosmotic flow on the electropherogram, but they are not separated by the technique. Another limitation of this study is that the CE used in this study can only detect species that absorb UV or visible light. Besides that, the AA concentrations in this research are higher than what is reported in water and wastewater samples. This is the case because most of the AAs have low absorptivities in the UV-visible region, and it was necessary to increase their concentrations to observe them.

Potential Constraints

A constraint on the project was that the molar ratios of Cl_2:AA being studied were only 0.5:1 and 1:1, much lower than reported water sanitation concentrations. The reasoning is that the study aimed to focus on the first steps of any AA chlorination mechanism. The importance of focusing on the initial steps lies in the possibility that those initial steps can dictate what occurs at later stages. We are viewing the beginning of the mechanism and I hypothesize that at

higher ratios, the products observed at lower ratios will continue to react. It is important to see what happens at the beginning. Future studies can focus more on the end of the mechanism and increasing the dosage of Cl.

CHAPTER TWO

LITERATURE REVIEW

Amino Acids Overview

Structures and Classification

Amino acids are the building blocks of proteins and larger organic molecules. The 20 essential AAs have distinct side chains that alter their reactivities. Structurally, the AA is comprised of its side chain, α-carbon, and the α-amine (Figure 1). The points being considered for the chlorine to react with are the side chains and the α-amine (Figure 1). Other than that, the AAs can broadly be categorized as polar, nonpolar, acidic, basic, and aromatic. The pK_a values of the AAs and the pH of the solution determine the relative charge of the AAs.

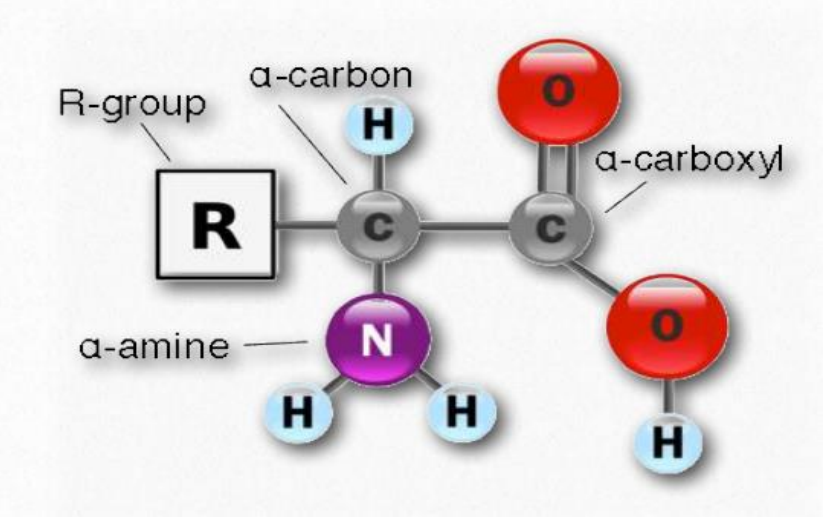


Figure 1. Amino Acid Structure.

Biochemistry Free For All. Structure and Function - Amino Acids

Biological Functions

AA synthesis in wastewater is most commonly due to the “decomposition of proteinaceous matter and by synthesis from inorganic matter from microorganisms” (Kanchanamayoon et al., 2015). AAs are paramount for all organisms including microorganisms such as cyanobacteria and algae, which are regularly found in wastewater. For that reason, past research suggests there is a link between algal blooms and AA concentrations. When an algal bloom occurs, AA concentration will rise in natural waters (Bond et al., 2011). This can result in a significant increase in DBP production rates to unsafe levels.

Chemical Reactivity

Chemical reactivity in AAs has everything to do with their structure. In a study done by Hong et al. (2008), it was found that amino acids with activated aromatic structures were potent trihalomethane (THM) precursors compared to other amino acids. For haloacetic acid (HAA) formation, amino acids containing active ring structures (OH- and nitrogen-substituted rings) were especially reactive to Cl_2. More chlorine is consumed with groups that contain double bonds in their structure and are more electron donating (e.g. -OH, -S, and -NH_2). The amino acids, all to an extent, exhibited high chlorine demand. However, despite not having a reactive ring structure, aspartic acid, and asparagine also produced high levels of HAAs (Hong et al., 2008).

Chlorination of Amino Acids

Mechanism and Pathways

The use of chlorination as a disinfectant in reclaimed wastewater is effective in eradicating microorganisms. The chlorine can dissolve in solution, and the resulting HClO disrupts important cellular functions (Bond et al., 2012). The reactions in the water are as follows:

$Cl_2 + H_2O \leftrightharpoons HClO + H^+ + Cl^-$

following an equilibrium of $HClO \leftrightharpoons H^+ + OCl^-$ is reached (Li et al., 2022). HClO is what primarily chlorinates and disinfects the water (Li et al., 2022). HClO, analogous to NaOCl, acts within bacteria to “damage the bacterial enzyme system” resulting in bacterial malfunction (Li et al., 2022).

What is known currently about the mechanism from amino acid to DBP is the AA to nitrile pathway (figure 2). With further chlorination of the nitrile, DBPs such as dichloroacetonitrile can be produced. For example, it was found that after chlorination of glycine, the reaction produced cyanogen chloride, CNCl (Na et al., 2006). These DBPs fall into the category of nitrogenous DBPs. There are two pathways Na and Olson pursued, by dehydration resulting in the formation of formaldehyde. Formaldehyde can easily be converted to HCN by further chlorination of CNCl (Na et al., 2006). This research will focus on the first and second steps of the reaction (figure 2), the mono and dichloroamine.

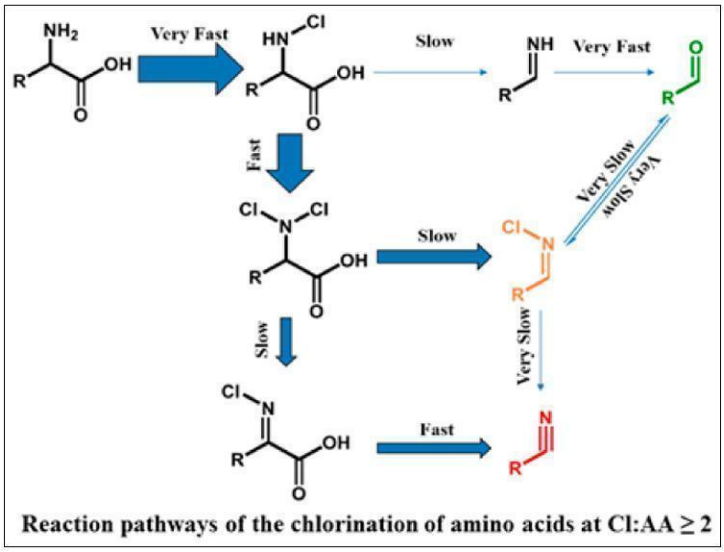


Figure 2. Amino Acid Chlorination Reaction Pathway. (*How et al., 2017)*

If the Na and Olson mechanism is applied here, after the first step the mono and di-chloroAAs will be produced. Afterward, the products will decompose, and that results in an N-chloroimine. That will then get hydrolyzed, and the resulting product will be an aldehyde. Another possibility is that only HCl is lost instead of H_2O, which will result in nitrile formation. The other deviation is if only the dichloro AA gets dechlorinated, if that is the case then the nitrile will be produced if the dichloro AA gets decarboxylated and then dechlorinated again. The paper's main finding is “stoichiometrically a 2:1 molar chlorine:glycine ratio will produce cyanide and a 3:1 ratio will produce cyanogen chloride.” (Stanley et al. 2022).

Factors Influencing Chlorination Disinfection

During wastewater treatment, the dose of chlorine can vary based on the "chlorine demand, wastewater characteristics, and discharge requirements" (EPA, 1999). The concentration dose varies from 5 to 20 mg/L (EPA, 1999). As shown in Figure 3, The wastewater passes through multiple processes until it reaches the Chlorine Contact Basin for Disinfection. After the initial chlorination, there is a dechlorination process that aims to remove the "free and combined chlorine residuals" (EPA, 1999). The most common dechlorinating chemicals are sulfur dioxide, sodium bisulfite, and sodium metabisulfite (EPA, 1999). Despite the dechlorination process occurring after chlorine disinfection, at that point, DBPs have already formed. The chemicals used to dechlorinate are not strong enough to remove entire DBPs. This is where better forms of DBP detection can be used to mitigate the potential damage DBPs can impose.

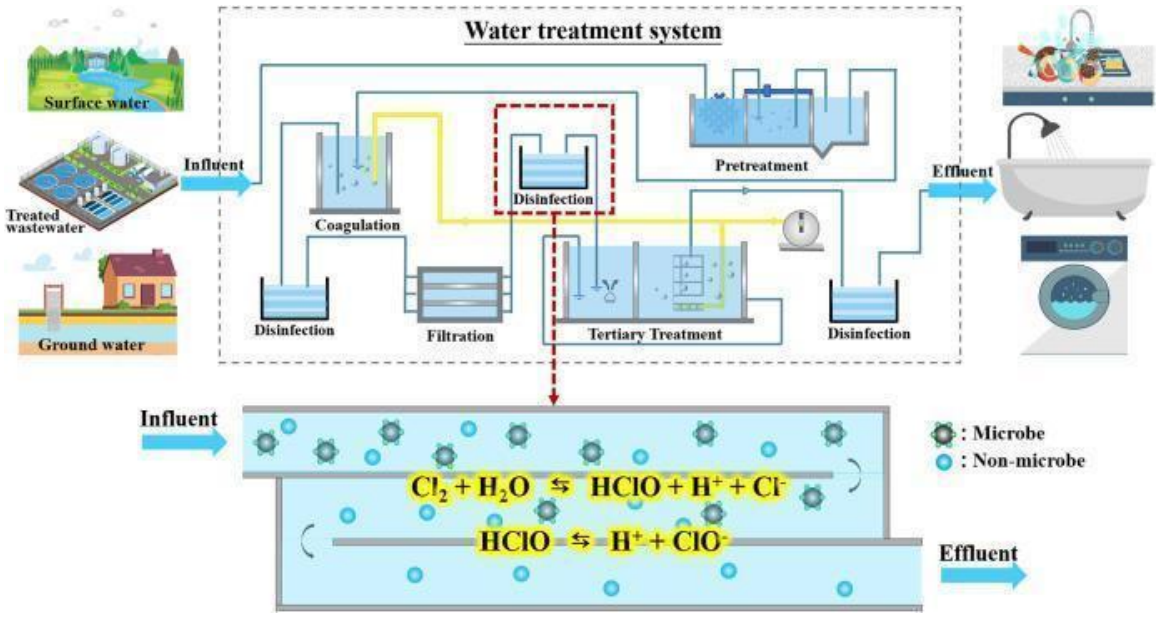


Figure 3. Chlorine Disinfection Process. (*Li et al., 2022*)

Biological Implications

The problem with this disinfection process is that the dissolved chlorine also has a proclivity to react with natural organic matter such as AAs, the product synthesized from that reaction will be a halogenated compound (Hong et al., 2008). DBPs are known water pollutants, the EPA regulates their concentration in drinking water. Biologically these compounds are toxic. DBPs like THM, $CHCl_3$, $CHCl_2Br$, and $CHBr_3$, are known as group B carcinogens (Mishra et al., 2014). Not only do they cause biological damage, but they can also be environmental pollutants.

Capillary Electrophoresis

Principles and Operation

Generally, the techniques used to analyze the chlorination reaction with amino acids are gas chromatography/mass spectrometry (GC/MS), high-performance liquid chromatography (HPLC), and CE. This study is interested in pursuing CE to analyze amino acids unreacted and during the chlorination reactions with increasing levels of chlorine. This will be the first time CE will be used in this manner. CE as a method for measuring amino acid reactivity is sound due to its ability to separate compounds based on their electrophoretic mobility. This works by applying an electrical field to the capillary, which is filled with a buffer, the electrical field applied will make the AAs migrate through the capillary. The rate at which they migrate depends on the AAs' electrophoretic mobility; smaller and more highly charged molecules will move faster than larger less charged ones (Ta et al., 2021). When the voltage is applied to the capillary the reaction mix is transported through the capillary by the surrounding cations in the capillary (Li et al., 2000). From this, the reaction mix will migrate toward the cathode's UV-Vis detector from the anode. What drags the entire solution forward is the water's cohesiveness, this, in turn, results in a flow across the capillary, or an electroosmotic flow (EOF) (Li et al., 2000). From that what is recorded by the

electropherogram will be the migration time vs absorbance. An example of each unchlorinated AAs electropherogram is found below.

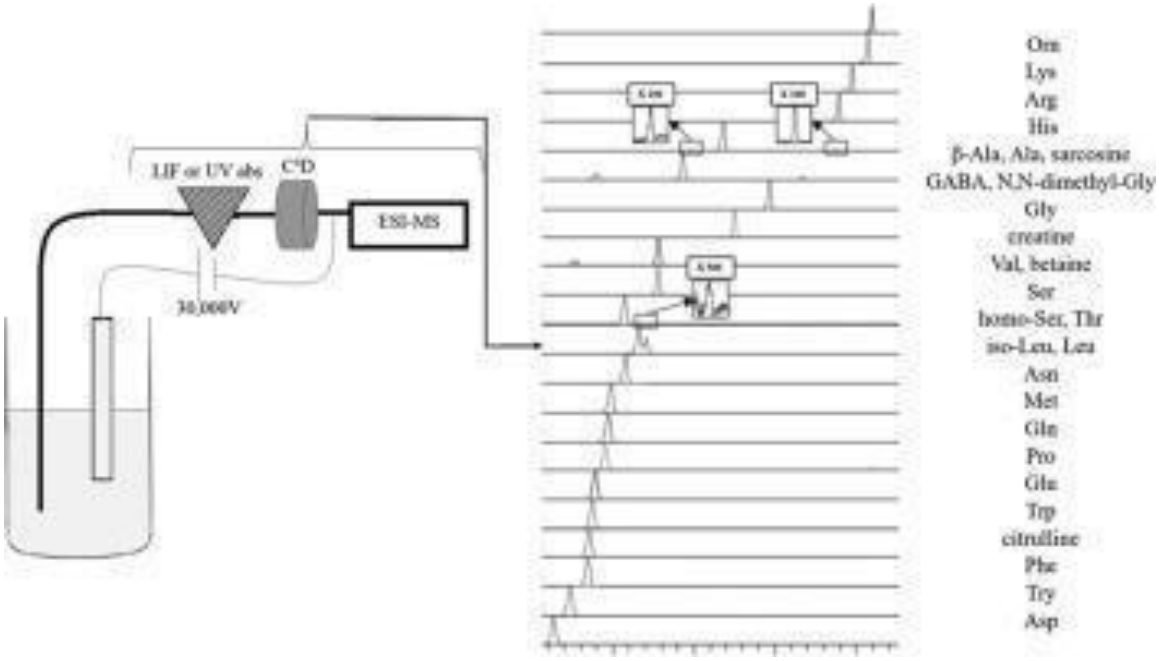


Figure 4. CE AA Electropherogram.

Ta et al, 2021

Applications in Chemical Analysis

What will be characterized are unreacted AAs, the DBP ions from the AA chlorination, and the neutral species. In this setting there are charged species, meaning DBP ions are detectable in theory but have yet to be shown with CE. Even so, a positive of the technique is that it can analyze multiple ions in one mixture, per AA. Once moved into the CE to analyze the AA reaction mix

contents, the samples are injected into a CE buffer, 0.01 *M* 9.2 pH sodium tetraborate. The higher pH of the buffers will end up deprotonating the ammonium group of the AA. This leaves us with a negatively charged ion, this will only occur if the pH is higher than the pI of the AA. As for the neutral species in the reaction, they accumulate and are visible in what is called the neutral zone.

Previous Studies Using CE

CE is a novel method for this type of analysis, it was chosen because it can separate at a high efficiency compared to a method like high-performance liquid chromatography (Manaenkov et al., 2003). This is innately due to electroosmotic flow (EOF), as is it a “simple and highly efficient way of driving a separation system” (Li et al., 2000). Furthermore, because of the “extremely narrow cylindrical region” of the capillary, the reaction mix is pushed out of the capillary with no pressure drop (Li et al., 2000). What is obtained from this ‘no pressure drop’ is “a plug-like flow”, a flow commonly associated with high column efficiency (Li et al., 2000). HPLC lacks this “plug-like flow” profile and instead, its own “pressure-based flow introduces a parabolic profile”, leading to a decrease in overall efficiency (Li et al., 2000).

Working with CE with UV-vis detection means it is necessary to monitor the AA at higher concentrations. However, it consumes fewer reagents, does not use high-pressure pumps, and is quicker than other methods with 15 minutes for an electropherogram (Manaenkov et al., 2003). For those reasons, using this method is also cost-efficient, a factor crucial for the execution of studies

(Manaenkov et al., 2003). If MS detection is available, CE-MS can be used to determine the identity of the DBP ions, e.g. the mono- or dichloroamines.

Gaps in Existing Literature

What is Known

Previous studies have been done on the AAs themselves, using CE as the method of analysis, but they have yet to be done on the chlorination of AAs. This study aims to determine the usefulness of CE in detecting separated DBP ions with the UV-vis detection available in our laboratory. Future studies through the combined use of CE-MS may then be implemented, where available, for qualitative analysis of the DBPs. The basis of our study is previous experiments done to rapidly determine AAs by CE without preliminary derivatization (Manaenkov et al., 2003). This is also important because the derivatizing agents will likely react with chlorine; thus this CE method will not generate its own DBPs. However, there were some deviations from Manaenkov's methods such as different concentrations. The concentrations used were 10, 50, and 100 *mM* of AA stock solutions. Another difference was that the capillary itself is smaller so our migration times, per AA, varied from theirs. Besides that variation, they did a mixture of AAs, whereas in this study it was one AA per CE. The Manaekov study provided a basis for the control experiments before the AA chlorination.

Areas that Require Further Investigation

An area that requires further investigation is the complete results of all AA chlorinations, the products are known for some AAs but not the mechanisms. For example, glycine's pathway to CN and cyanogen halide is known but not how other AAs result in those same products (Stanley, 2021). That is a gap this research can plug with the introduction of CE analysis. Moving onto DBPs, they have only been studied for about 40 years, the major concerns are THM, HAA, and HANs. About 600-700 DBPs have been researched, coming from not only chlorine but also chlorine dioxide, chloramines, and ozone (Kanchanamayoon et al., 2015).

AA reactivity changes with the addition of chlorine. A previous study found that amino acids with aromatic rings were precursors for trihalomethanes and HAAs were found to come from active ring structures (Hong et al., 2008). The study also found that in general amino acids will produce higher amounts of HAA compared to trihalomethanes (Hong et al., 2008).

CHAPTER THREE

METHODOLOGY

Methods

Our sample collection started with making 10 *mM*, 50 *mM*, or 100 *mM* solutions of each amino acid in DI water. Some AAs needed to be more concentrated for a clearer electropherogram. When testing with NaOCl the amino acids will be diluted with a phosphate buffer. The purpose of this is to maintain a steady 7 pH within the solution so relative charges for the AAs and DBPs stay constant throughout the reactions. All AAs except for arginine, lysine, and histidine have pI values less than 7 and are thus negatively-charged at this pH. During CE with the borate buffer at pH = 9.2, all AAs are negatively-charged except for arginine and lysine. The CE instrument conditions are found in figure 5.

Voltage Max kV	30
Current Max µA	300
Cartridge Temperature °C	22
Sample Storage °C	22
Peak detect parameters Threshold	2
Peak Width	9

Figure 5. CE Initial Conditions. Voltage and temperature information.

1. CTRL solution = 10 mM AA + 10 mM KH2PO4 Buffer + 100 mM Ethyl Acetate
2. 1:0.5 Cl RXN = 10 mM KH2PO4 buffer + 10 mM AA + 12.7 uL NaOCl
3. 1:1Cl RXN = 10 mM KH2PO4 buffer + 10 mM AA + 25 uL NaOCl

Spin for 1 min, run 1 mL of RXN solution through CE. Continue to let RXN spin for 4 hrs

Make 1:0.5 Cl RXN mix: 45 mL In 50 mL amber bottle

Yes

Make + Run CTRL solution though

Is AA peak large enough? No contaminants?

Scale Buffer + AA + Cl2 to either 50 or 100 mM

No

Increase CTRL mM to 50 mM or 100 mM

Run new CTRL solution through CE

If electropherogram is clean, continue with chlorination

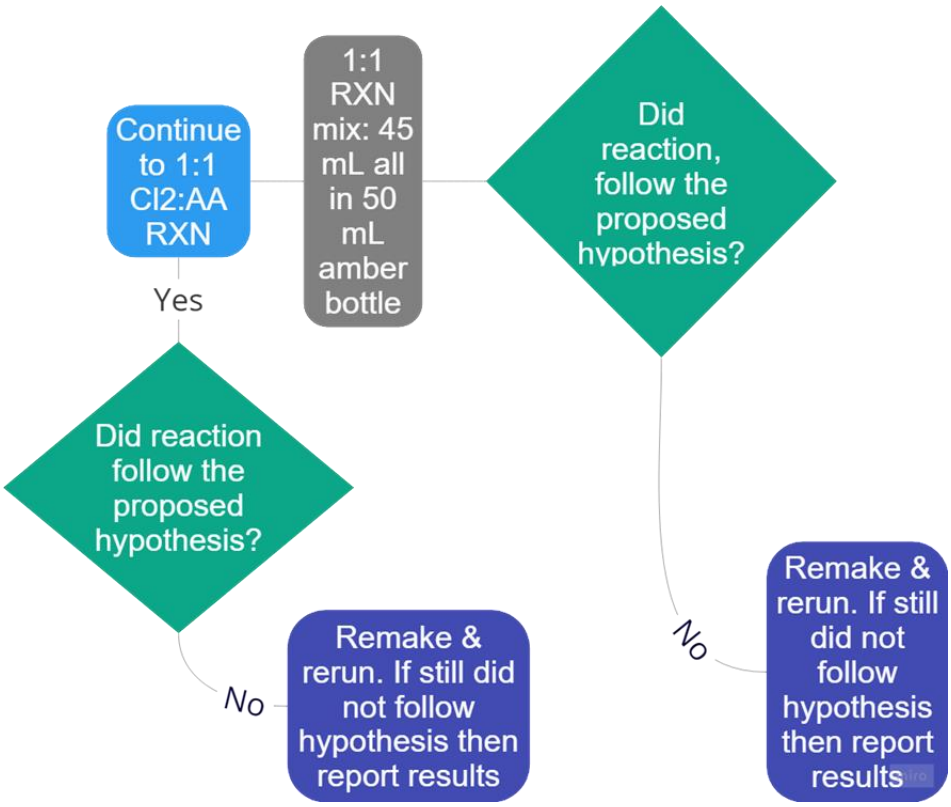


Figure 6. AA Chlorination Methodology.

The complete methodology for running the chlorination reactions can be found in Figure 6. To begin, our methodology begins by preparing a control solution of unreacted AA. This control solution will consist of 1 mL of the stock AA solution, 1 mL of 100 mM ethyl acetate, and 8 mL of the KH_2PO_4 buffer (pH = 7). The ethyl acetate serves as a neutral zone marker. The control solution will be run through CE, the time program for which consists of 6 steps. These 6 steps included rinsing with water, cleaning with NaOH, conditioning with the borate buffer, injecting the sample, and then applying a 23 kV electric field (Figure 7).

During this, the capillary is cleaned with NaOH, and then the capillary is conditioned with the "running" buffer before sample injection. The capillary ends remain immersed in the sodium tetraborate buffer throughout the separation. The entire analysis lasted 19.5 minutes. A photodiode array, PDA, detector was used; the scanning range was 190-300 nm and all electropherograms were analyzed at 200 nm.

Time Program						
Event	Value	Duration	Inlet Vial	Outlet vial	Summary	Description
Rinse - Pressure	25 psi	1 minute	B1:C1	B0:B1	Forward	water in B1:C1
Rinse - Pressure	25 psi	1 minute	B1:D1	B0:B1	Forward	NaOH in B1:C1
Rinse - Pressure	25 psi	1 minute	B1:C1	B0:B1	Forward	water in B1:C1
Rinse - Pressure	25 psi	1.5 minute	B1:B1	B0:B1	Forward	water in B1:C1
Inject - Pressure	0.5 psi	9 sec	S1:D1	B0:B1	Override, Forward	buffer in B1:C1
Separate - Voltage	23.0 kV	15 min	B1:A1	B0:A1	0.17 Min ramp, normal polarity	B1:A1, B0:A1
Stop Data						

Figure 7. CE Time Program. these are the conditions the solution is put through before the analysis.

The chlorination reactions took place in 50 mL brown flasks. Due to the possible photosensitivity of the reaction the flasks were capped with black caps. 45 mL of the reaction mixture will be 9 mM 7 pH phosphate buffer, 5 mL will be the 10 mM AA, and depending on the Cl_2 dosage either 12.7 uL (for 0.5:1 reactions), or 25.4 uL (for 1:1 reactions) of NaOCl solution was added. The calculation for determining the amount of Cl to add to the solution can be found in Figure 8. The stock bottle of NaOCl was at a concentration of 2.46 *M* or 1.96 *M*, and the NaOCl concentration was targeted to be 0.5 *mM* for the 0.5:1 reaction and 1.0 *mM* for the 1:1 reaction (Figure 8).

$$0.05\,L\,Total\,Volume \times 0.001\,\frac{mol}{L}\,Target\,AA\,Concentration = 0.0005\,moles$$

$$\frac{0.0005\,moles}{2} = 0.000025\,moles\,due\,to\,\frac{1}{2}Cl:AA$$

$$\frac{0.000025\,moles}{1.963\,M\,of\,Cl} = 0.0000127\,L \times 1000\,mL/1\,L = 0.0127\,mL$$

$$For\,a\,1\,Cl:1\,AA\,reaction\,the\,calculation\,is\,as\,follows:\ \frac{0.00005\,moles}{1} = 0.0005\,moles$$

$$\frac{0.00005\,moles}{1.963\,M} = .000025\,L \times 1000\,mL/1L = .025\,mL$$

Figure 8. Chlorine Dose Calculations. For 0.5:1 - 1:1 Cl_2:AA

After running the control solution in the CE 1 mL was drawn from the 50 mL reaction flasks and put into a vial then run through the CE. The next step was

to determine if there is any time dependence of product formation, which will be determined by taking an electropherogram of the reaction solution first at the 1-minute mark and then hourly for 4 hours total. The reaction was stopped when the product peak became stable, meaning it did not grow or decrease in size. The peaks stabilized in the amino acids that only went for less than 4 hours (Tables 1,2). On the contrary, if the main product peak disappeared before 4 hours, the reaction was stopped. This Pure AA CE preparation was adapted from a previous study from Manaenkov. Concentrations and volumes were adjusted for minimal background in the electropherogram, 10 mM, 50 mM, and 100 mM. All results are recorded in Excel, and then graphed by groups, the groups are AA, product, neutral zone, and dose comparison.

Our treatment of amino acids with NaOCl will range from a 0.5:1 molar ratio to a 1:1 molar ratio (Cl_2: AA). If the chlorination reaction is occurring, as the chlorine concentration increases the size of the amino acid peak will decrease. Other than that, it is expected that there will be more products, or peaks, on the electropherogram. Work will be done in future studies to qualitatively analyze the products of interest, i.e. the DBPs such as HCN. At lower chlorine ratios, the initial products expected are N-chloro amino acids, which should be similarly ionic and thus will separate at their own unique migration time. These initial products may react further with increased chlorine and/or time to form neutral DBPs. Future analysis of the products will be attempted utilizing MS. The

purpose of this study is to document the presence of DBP ions as CE peaks that form upon chlorination of the 20 AAs.

CHAPTER FOUR

RESULTS

Presentation of Data

Chlorination of All Amino Acids

AA	Apparent Mobility (μm·cm/V·s)	Response Factor (M)	AA Peak (Min)	Initial Height	Final Height	Fraction remaining AA Final/Control (%)	AA Rxn (Hrs)
Threonine	4.95×10^{-8}	18763000	4.175	18763	16549	88.20	4.00
Histidine	4.95×10^{-8}	16441000	4.175	16441	9961	60.59	4.00
Methionine	4.55×10^{-8}	30593000	4.542	30593	17884	58.46	4.00
Glutamic Acid	3.02×10^{-8}	7494000	6.833	7494	4098	54.68	4.00
Aspartic Acid	2.84×10^{-8}	4432000	7.275	4432	4397	99.21	3.00
Asparagine	3.94×10^{-8}	15513000	5.242	15513	16654	107.36	3.00
Arginine	6.63×10^{-8}	25585000	3.117	25585	13773	53.83	4.00
Tyrosine	4.92×10^{-8}	444850000	4.2	444850	108340	24.35	3.00
Isoleucine	5.03×10^{-8}	1342000	4.108	1342	1266	94.34	3.00
Lysine	5.51×10^{-8}	5729200	3.75	28646	14922	52.09	3.00
Proline	5.58×10^{-8}	581000	3.7	5810	6185	106.45	3.00
Serine	4.24×10^{-8}	4673000	4.867	4673	2474	52.94	3.00
Glycine	5.35×10^{-8}	111500	3.858	1115	1078	96.68	4.00
Cysteine	3.68×10^{-8}	22185000	5.617	22185	6510	29.34	3.00
Leucine	5.07×10^{-8}	1246200	4.075	6231	5791	92.94	3.00
Alanine	5.08×10^{-8}	4662000	4.067	4662	4197	90.03	4.00
Valine	5.45×10^{-8}	3518000	3.792	3518	1636	46.50	3.00
Glutamine	4.42×10^{-8}	16731000	4.675	16731	10960	65.51	3.00
Phenylalanine	5.03×10^{-8}	111412000	4.108	111412	4.158	59.07	3.00
Tryptophan	4.78×10^{-8}	144537000	4.325	144537	4.342	97.64	3.00

Table 1. 0.5:1 AA peak chlorination results. AA peak heights, rxn time, and MTs.

Table 1 introduces what occurs to the AA peak whilst it is being chlorinated. The AA peak migration time range was 3-6 minutes. The neutral zone for uncharged species was at 3.5-3.7 minutes. Peak height varied with amino acid; the visual trend was the R group was the deciding factor in peak height. For example, AAs that had an R group such as an aromatic ring chromophore naturally had larger peaks. Peak height was also detected by UV-Vis absorbance, 214 nm. Besides molecular weight, the amino acid's charge in a pH 9 buffer also skewed the migration time.

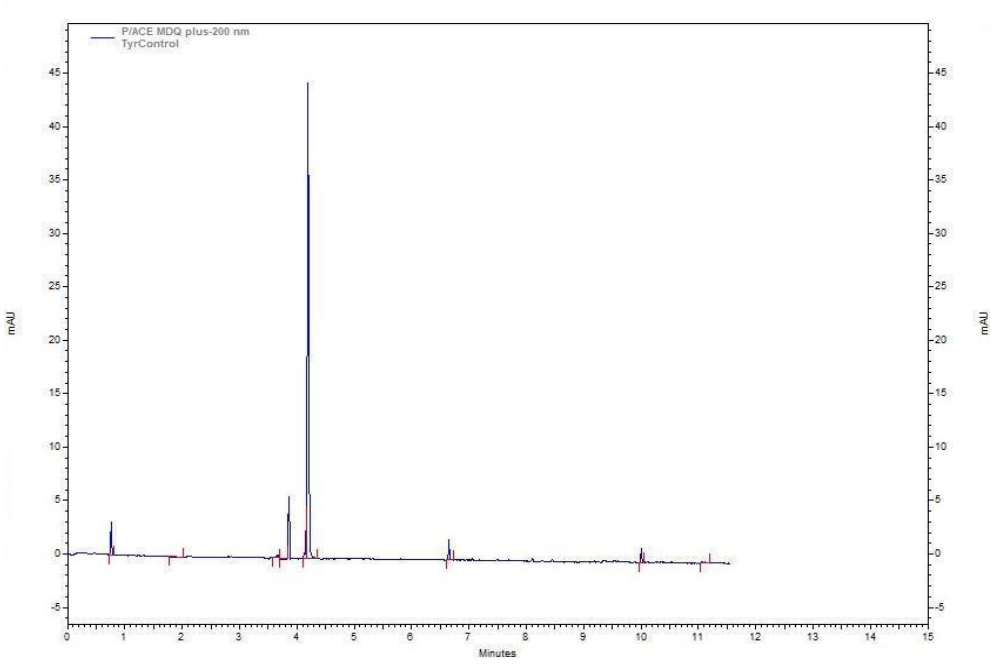


Figure 9. Tyrosine Electropherogram. Tyrosine Control showing its peak height.

The AAs all exhibited 3 types of peaks, the AA peak, the neutral zone peak, and the product peak. The most prominent peak was the AA peak for all AAs. The main product peak is generally in the 4–7-minute range. The height of the peak for both AA and product depends on the side chain of the AA itself, AAs like tyrosine and phenylalanine had huge products and AA peaks (Figure 9). This is due to them having aromatic chromophores. Besides side chain variability concentration was also used to increase AA peak size to a viewable absorbance. From both chlorination reaction doses, it can be surmised that as the reaction goes on the product and AA peak will both decrease (Figure 11). However, this trend was not always the case, some AAs had their peaks stagnate in height while their product peak fluctuated, or vice versa (Figure 11). It was determined if the fraction remaining was over 100% then that showed a stagnation in growth (Figure 11). All reactions were 1-4 hours. The reaction was over when both the AA peak and DBP ion peak were both stable.

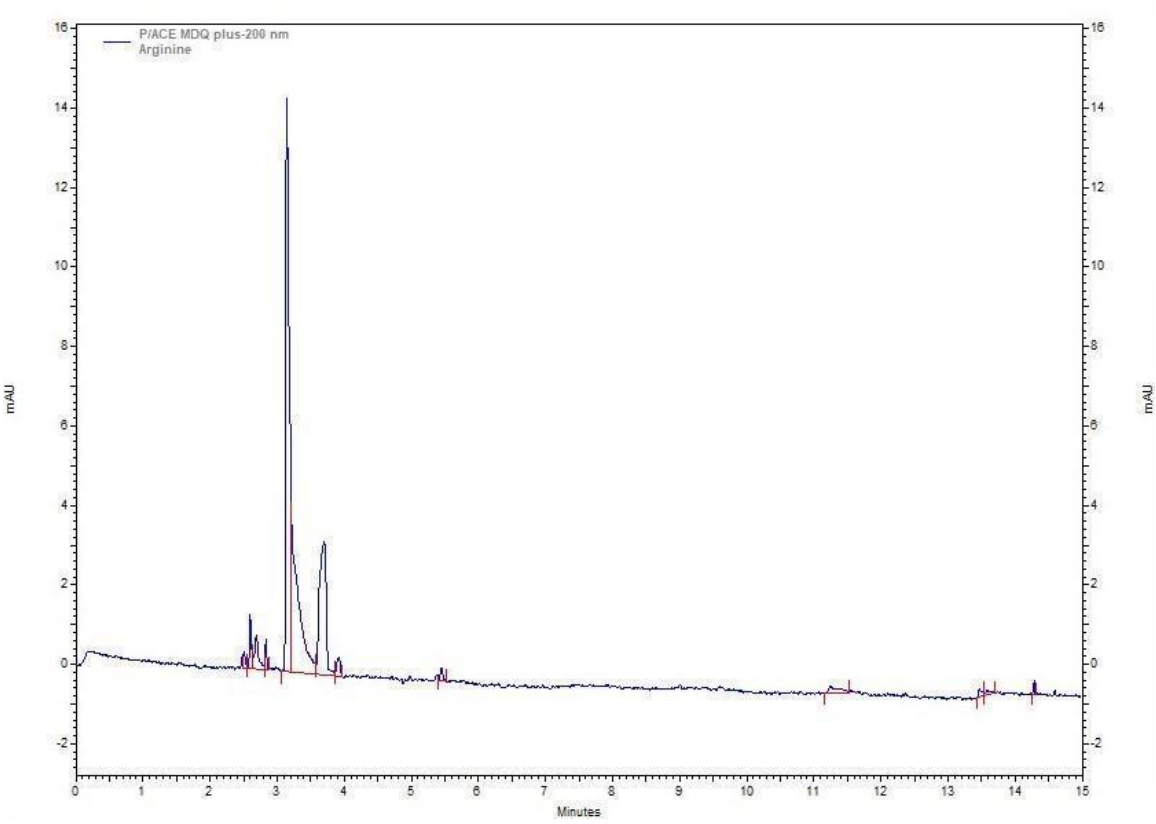
Another thing to note was that the 1:1 had far more products than the 0.5:1 reactions. This is due to the increase in Cl_2, as concentration increased the reaction was fueled. The increase in products did make the overall electropherogram harder to read as there were many new peaks to account for. The first product peak observed as the largest peak in the 0:5:1 reactions after 1 minute was compared to its size after the full reaction time of 1-4 hours, as well as to its size in the 1:1 reactions. Besides migration time, the peak also had to follow the decreasing peak size over time trend. Besides there being more

products in the 1:1 reactions there were new products as well. Based on the electropherograms Threonine (Thr), Methionine (Met), Aspartic Acid (Asp), Proline (Pro), and Cysteine (Cys) all had new main product peaks (Table 2,3). The peak for Thr was at 4.73 minutes at 0.5:1 and at 1:1 it shifted to 6.28 minutes. Met started at 5.18 minutes and shifted to 7.17 at 1:1. Asp shifted from 7.93 minutes to 7 minutes. Pro shifted from 8.74 minutes to 6.78 minutes. Then Cys shifted from 5.63 minutes to 8.55 minutes. The product peak listed in Table 2 was the largest observed peak.

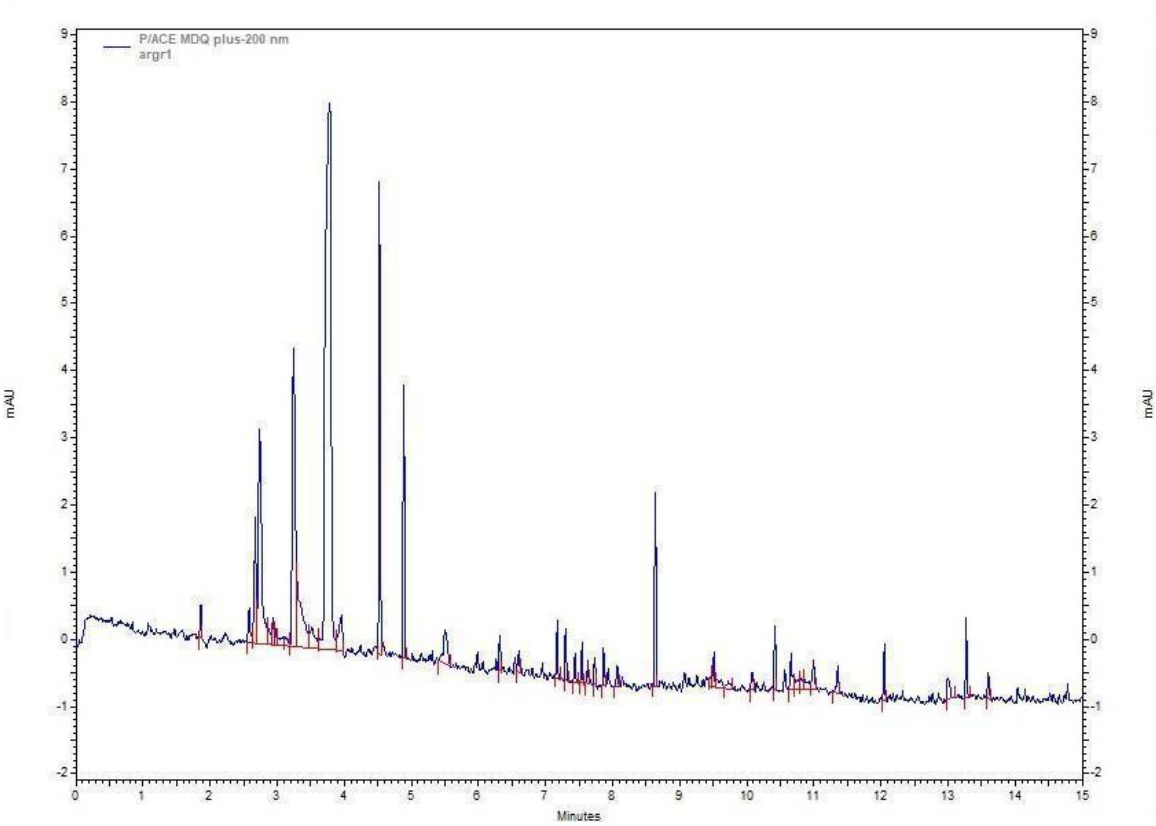
AA	Product Peak 1:1 (min)	Height	End Mt (Min)	End Height	Apparent Electrophoretic Mobility(μm·cm/V·s) Product	AA rxn Hrs	Product rxn hours
Threonine	6.28	4,001	6.33	1,488	3.14×10^{-8}	3.00	3.00
Histidine	5.73	21,145	5.72	2,114	3.61×10^{-8}	2.00	2.00
Methionine	7.17	3,044	7.23	1,245	2.88×10^{-8}	2.00	2.00
Glutamic Acid	No Peak					4.00	
Aspartic Acid	7.00	4,668	6.99	4,455	2.95×10^{-8}	2.00	2
Asparagine	5.98	5,991	5.92	213	3.45×10^{-8}	3.00	3.00
Arginine	3.82	8,940	3.75	1,359	5.41×10^{-8}	3.00	3.00
Tyrosine	5.96	67,595	5.98	14,093	3.47×10^{-8}	3.00	3.00
Isoleucine	5.90	4,940	5.81	2,205	3.50×10^{-8}	2.00	2.00
Lysine	No peak					4.00	
Proline	6.78	2,132	6.68	2,315	3.05×10^{-8}	3.00	3.00
Serine	6.53	5,991	6.53	5,991	3.16×10^{-8}	2.00	2.00
Glycine	6.63	17,488	6.71	9,101	3.11×10^{-8}	2.00	5.00
Cysteine	8.55	1,929	8.62	419	2.42×10^{-8}	4.00	4.00

Leucine	5.71	2,634	5.67	574	3.62×10^{-8}	3.00	2.00
Alanine	6.78	8,536	6.69	998	3.05×10^{-8}	STABLE	4.00
Valine	5.83	3,704	5.76	1,578	3.54×10^{-8}	2.00	2.00
Glutamine	5.86	6,991	5.72	2,291	3.53×10^{-8}	3.00	3.00
Phenylalanine	5.67	38,299	5.64	2,097	3.64×10^{-8}	4.00	3.00
Tryptophan	5.31	19,511	5.31	4,616	3.89×10^{-8}	2.00	2.00

Table 2. 1:1 Cl_2:AA Product Peaks Data.



0.5:1 Arginine at 1 minute of chlorination



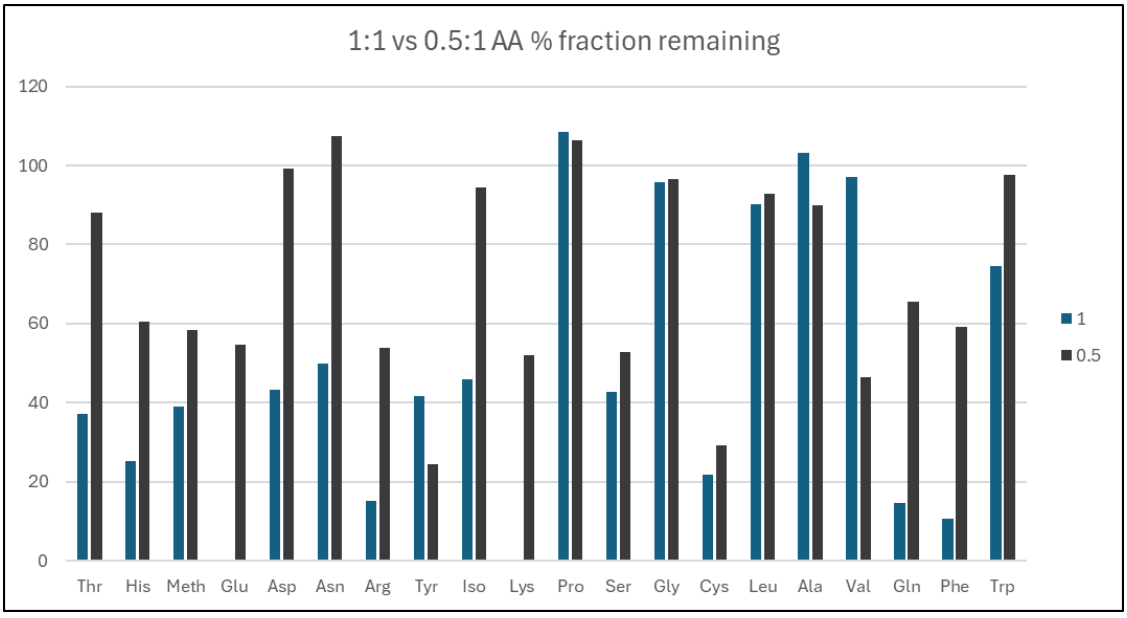
1:1 Arginine at 1 minute of chlorination. All peak after 4 minutes are new.

Figure 10: Electropherogram Difference Between Dose. the electropherograms show the increase of significant peaks as the dose of Cl_2 increases.

The analysis consisted of grouping the equal trials and noting the approximate uncertainty in the migration times of the AAs and neutral marker. For a given batch of reagents, the uncertainty of the migration times was less than 0.1 min. If a shift in migration time was greater than 0.2 min, it was considered a different ion.

AM, % reacted, and RF

Besides using statistical tests to shed more light on the reactions, apparent mobility (AM), percent AA reacted, and the response factor was calculated. The electrophoretic mobilities were calculated from the reaction's product and AA peak. For the 0.5:1 reaction, the AA with the highest apparent mobility was Arginine at 6.63×10^{-8} (μm·cm/V·s), the lowest was Aspartate at 2.84×10^{-8} μm·cm/V·s (Table 1). All AAs in the 0.5:1 reaction were in the 2.84×10^{-8} to 6.63×10^{-8} μm·cm/V·s range. The product peaks, for the 0.5:1 reaction, were in the range of 2.36×10^{-8} to 5.57×10^{-8} μm·cm/V·s (Table 3). The product peak with the lowest electrophoretic mobility was from Proline while the highest was from Arginine (Table 2).



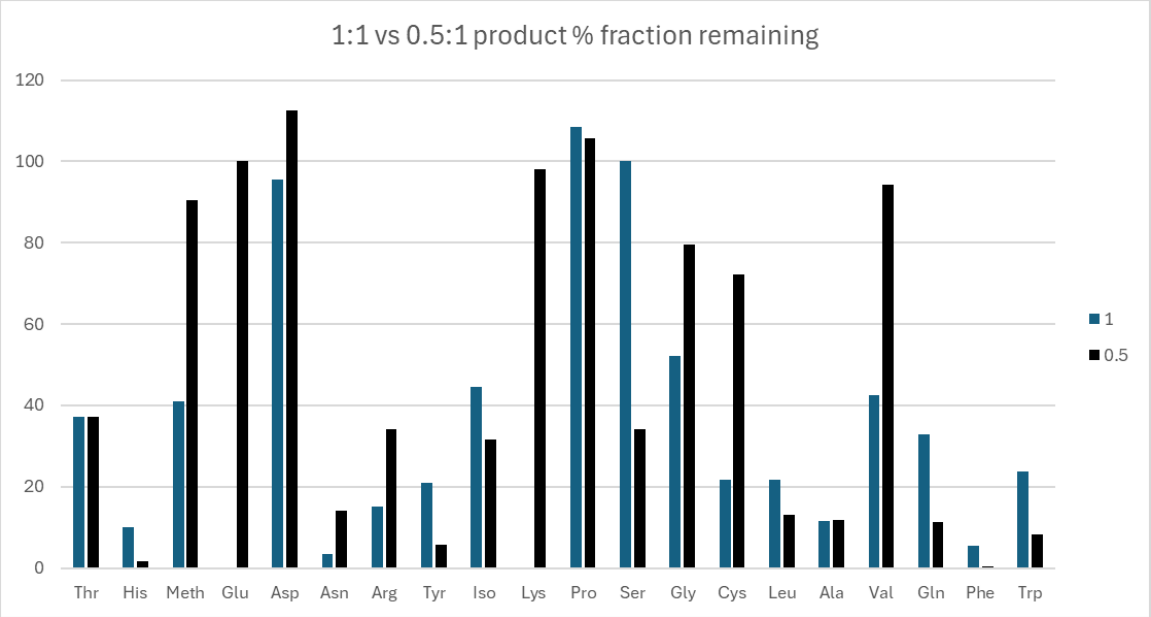


Figure 11. AA and Product Peak % Remaining after Reaction. Graphs quantify the decay of each peak for each AA throughout the reaction.

The fraction of the AA and product peak remaining after the entire reaction was calculated by dividing the peak's final height by the control's height (Figure 11). For the 0.5:1 trials the AAs that had their AA peaks stay at near 100% fraction remaining were Asp, Asn, Trp, and Pro. For the 1:1 trials, it was Pro and Ala (Figure 11). As for the product peaks for the 0.5:1 trials it was Glu, Asp, Lys, and Pro where this occurred (Figure 11). For the 1:1 trials, it was Pro, and Ser (Figure 11).

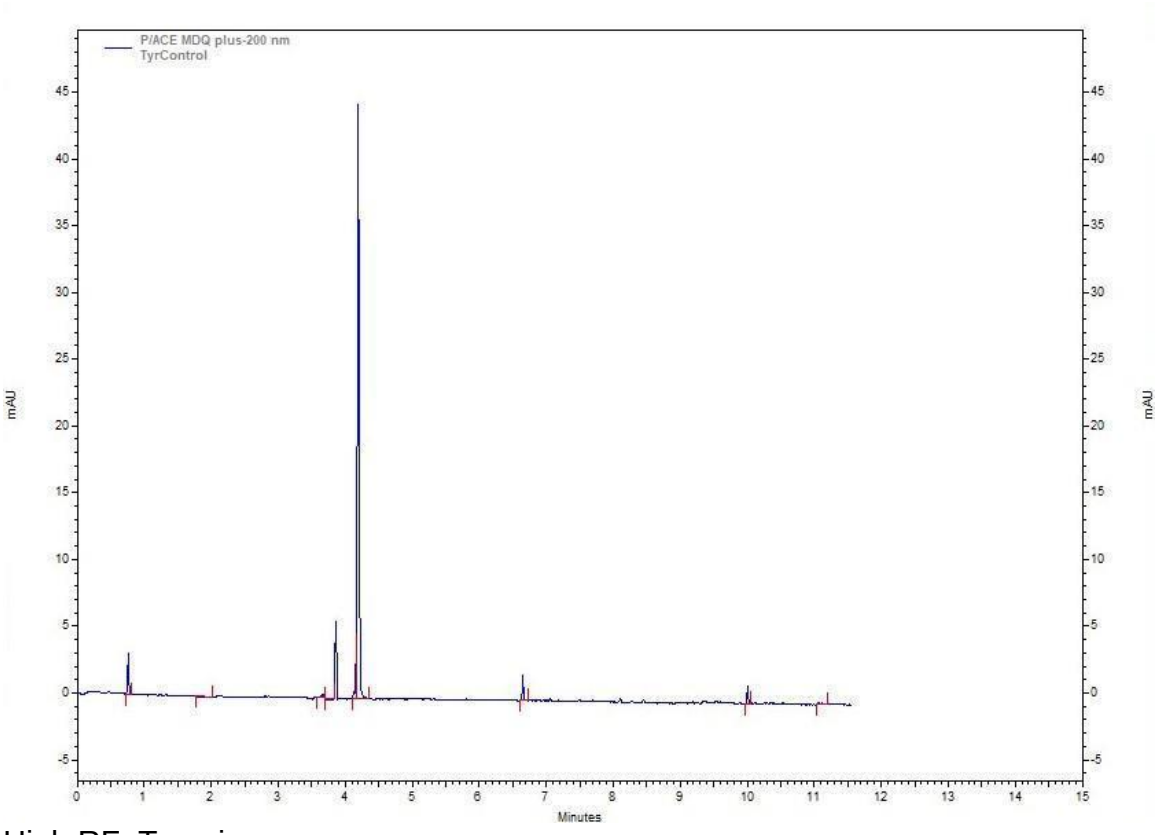
As seen in the data as the concentration of NaOCl increased the RF values decreased visually which can be seen in the electropherograms as the 1:1

trials had more products and more noise than the 0.5:1 trials (Table 2,3). Visually this can be seen in Figure 12 as the “Medium” RF electropherogram exhibits some noise but you can still distinguish the peaks from the noise. But if you look at a low RF electropherogram from Gly it gets increasingly difficult to tell the noise from the peaks apart (Figure 12).

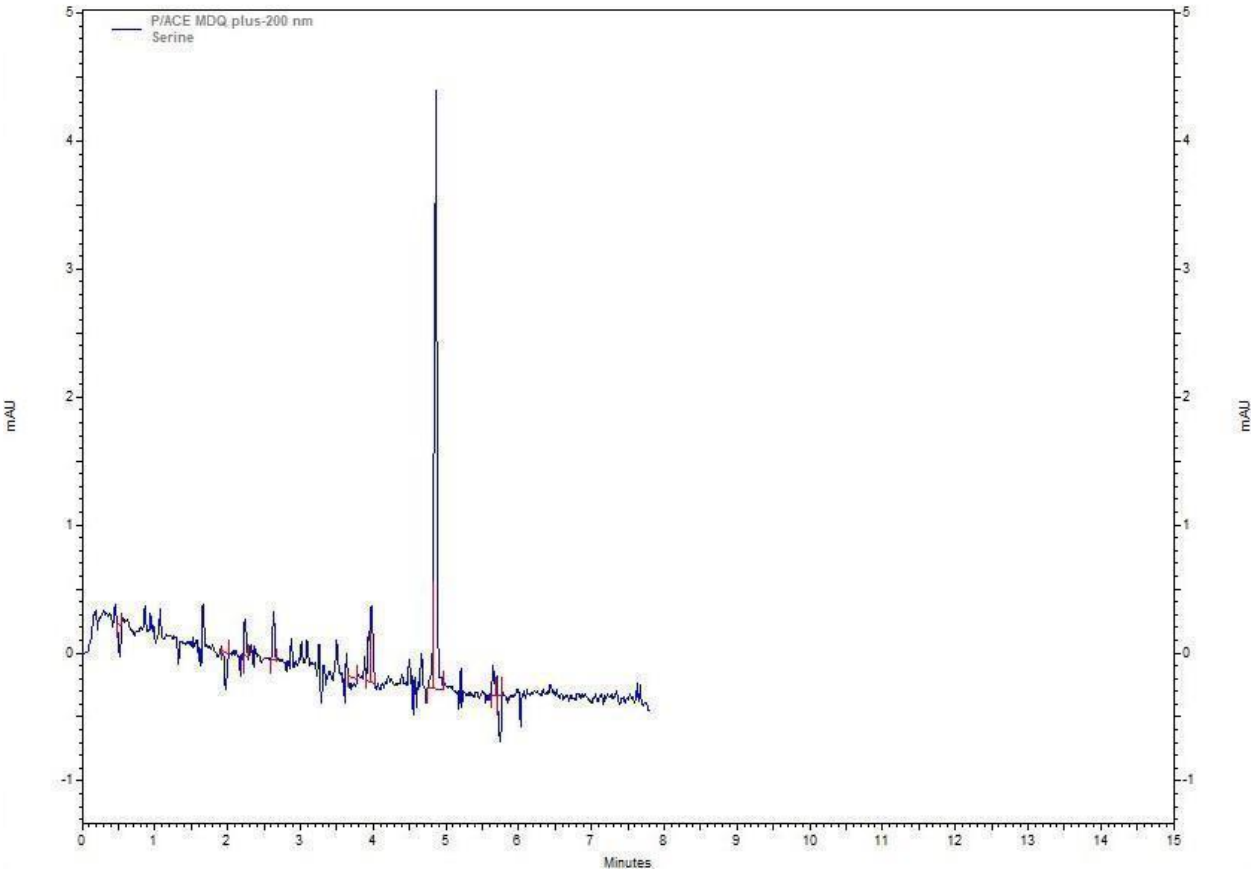
0.5:1 AA	RF Final (M)	Apparent Electrophoretic Mobility Product (μm·cm/V·s)	Main Product Peak (min) 0.5:1	Initial Height (1 min)	Final height	Total Reaction time (Hrs)
Threonine	18763000	4.37×10^{-8}	4.73	5,712.00	2,125.00	4.00
Histidine	16441000	3.52×10^{-8}	5.86	17,913.00	297.00	3.00
Methionine	30593000	3.99×10^{-8}	5.18	2,364.00	2,141.00	3.00
Glutamic Acid	7494000	2.84×10^{-8}	7.27	338.00	338.00	4.00
Aspartic Acid	4432000	2.61×10^{-8}	7.93	760.00	855.00	3.00
Asparagine	15513000	3.22×10^{-8}	6.41	3,146.00	445.00	1.00
Arginine	25585000	5.57×10^{-8}	3.71	3,353.00	1,144.00	4.00
Tyrosine	444850000	3.88×10^{-8}	5.33	34,302.00	1,962.00	3.00
Isoleucine	1342000	3.47×10^{-8}	5.95	1,682.00	530.00	3.00
Lysine	5729200	3.52×10^{-8}	5.87	2,772.00	2,717.00	3.00
Proline	581000	2.36×10^{-8}	8.74	688.00	727.00	3.00
Serine	4673000	3.01×10^{-8}	6.86	3,010.00	1,031.00	2.00
Glycine	111500	2.93×10^{-8}	7.05	4,597.00	3,662.00	4.00
Cysteine	22185000	3.67×10^{-8}	5.63	5,066.00	3,655.00	2.00
Leucine	1246200	3.47×10^{-8}	5.95	2,440.00	317.00	2.00
Alanine	4662000	3.03×10^{-8}	6.83	2,649.00	310.00	3.00
Valine	3518000	3.75×10^{-8}	5.50	3,615.00	3,407.00	2.00

Glutamine	16731000	3.40×10^{-8}	6.08	2,195.00	249.00	3.00
Phenylalanine	111412000	3.80×10^{-8}	5.43	34,963.00	128.00	8.00
Tryptophan	144537000	3.62×10^{-8}	5.71	3,199.00	263.00	3.00

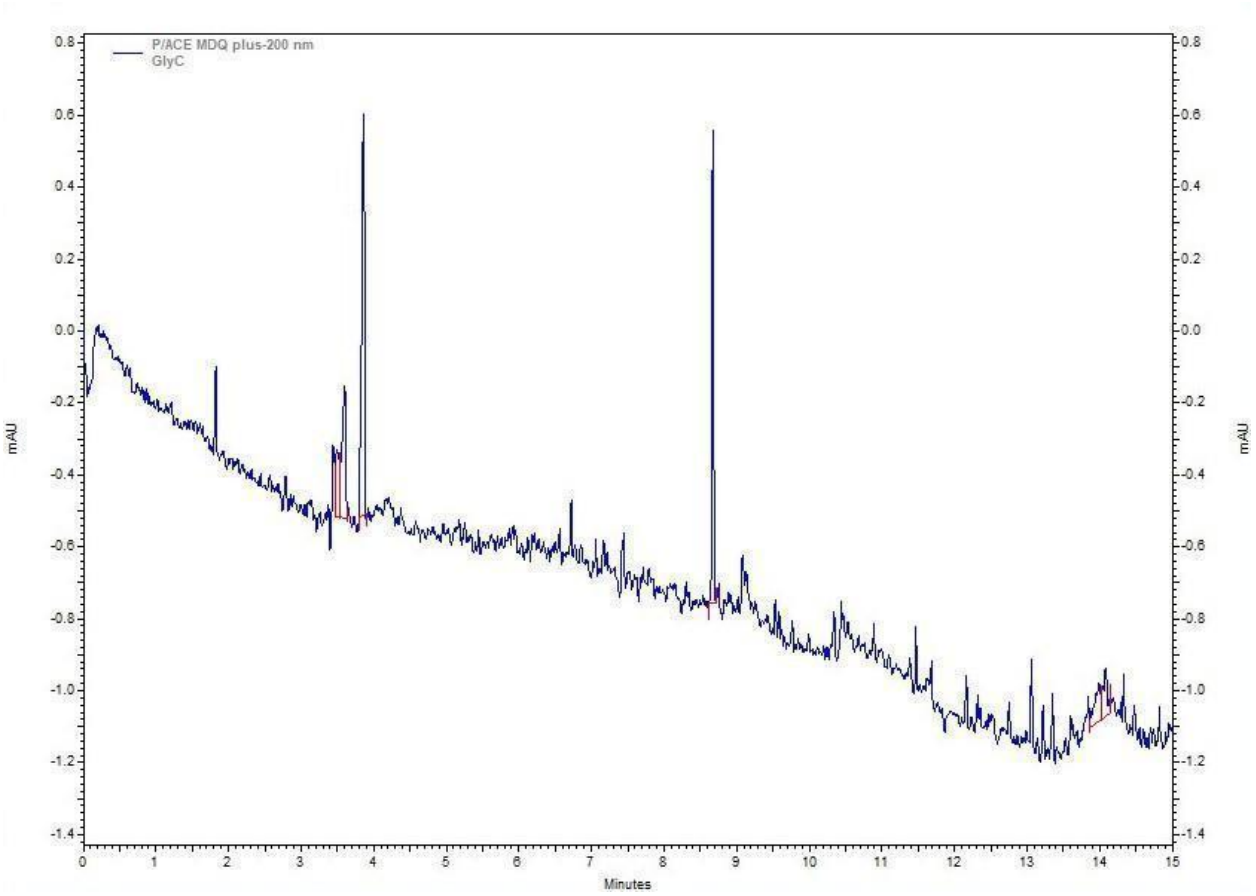
Table 3. 0.5:1 AA Product Chlorination Results. Includes RF and apparent electrophoretic mobility.



High RF: Tyrosine



Medium RF: Serine



Low RF: Glycine

Figure 12: RFs and Electropherograms. The electropherograms listed are that of a high RF AA (Tyr), medium RF AA (Ser), and a low RF AA (Gly).

CHAPTER FIVE

DISCUSSION

Interpretation

Comparison with Hypothesis

Within these trials, it was found what molar concentration of chlorine is most reactive with which amino acids, and which if the AAs will react similarly. This new insight has shed light on the mechanism of reaction. What was learned is that CE can be a reliable detector for DBP ions. Besides that, it was also determined that increasing the NaOCl dose will change the products of AA chlorination. From our trials, we know that the AAs will not all react similarly when chlorinated. The results here will aid in developing a new analytical method for assessing amino acid reactivity to chlorination.

Resulting Conclusions

Based on both dose trials the reaction will yield a neutral zone peak, an AA peak, and one or more product peaks. Previously it was hypothesized that the dose of chlorine would not change the reaction products. The 0.5:1 ratio trials would produce a monochloro amino acid, and the 1:1 trials would complete this reaction producing more of the same monochloro amino acid. The previous hypothesis was found to be false as the 1:1 trials had different product peaks and significantly more of them than the 0.5:1 trials. The peaks are different because they have different migration times and heights. Increasing the dose of Cl also increased the number of products by an average of five.

The AAs that did not have the same product peak were Thr, Meth, Glu, Asp, Pro, and Cys. Meaning their migration times shifted when the dose increased. Overall, there were significantly more products at 1:1 than at 0.5:1, it suggests the R groups are reacting early on, whereas the literature suggests later at higher Cl:AA ratios.

The product peaks for both doses must be DBP ions due to their migration times being different than the neutral time zone. During the reaction, the product peak decreased in height as the reaction carried forward. This means the product is continually reacting with the NaOCl or decomposing into additional products which falls in line with how DBPs react with a Cl dose in the general mechanism proposed in the literature.

Besides the reaction pattern, the migration time of the proposed products directly correlates with where ions would appear on an electropherogram. The migration times for the products reported were in the range of 3.71 to 8.55 minutes and the neutral zone in our reactions is at 3.26 minutes. Anything before the neutral zone is a positive ion while anything after it is negative. Seeing as all our products are in the negative zone, that falls in line with the charge expected from amino acid chlorination. The mono- or dichloro amino acid will carry an overall negative charge at pH 9.2 due to the Cl bonding to the alpha amine. However, other DBP ions resulting from these may also carry a negative charge at this pH. Another thing corroborating the charge of the products is their

apparent mobility; across the board the apparent mobility was higher for the products. That indicates the products are more negatively charged than the AAs.

The lower RF values for products observed in the 1:1 reactions suggest that initial products formed in the 0.5:1 reactions may have decomposed further, their chromophoric functional groups being reacted. As they, the chromophores, are being reacted more and more during the 1:1 reactions then they will further decompose. The AA chlorination products are DBP ions because they exhibit the correct time profile and reaction behavior. The increase in migration times indicates that either the pK_a values have decreased and/or the size of the ion has decreased. If the pK_a decreased, the negative charge would increase, and electrophoretic migration would increase towards the anode thus retarding its apparent migration time. If the size of the product decreases, this apparent migration in the opposite direction is amplified. CE is now proven to be able to track AA chlorination reactions for all 20 AAs.

The reactivity patterns noted from the chlorination were that the acids had weak reactions as seen by their AA peak not decreasing in size as was initially expected. As seen with Glutamic Acid, during its 1:1 trial there was no product peak, the only visible peak was its AA peak. Even so moving from the control to the 1 minute trial the migration time moved from 6.617 to 6.417 minutes. Following that at four hours the peak was at 6.45. Throughout the entire reaction the peak height went from 2934 to 1619, less than a 50% decrease.

It seems that in both NaOCl doses, the AA peak was stable over the full course of the reaction after decreasing initially in a 1–5-minute time frame. However, the five AAs that had shifted products appear to have highly electronegative and nucleophilic side chains. Specifically, Cys and Met both have sulfur in their side chain that could explain their shift of product migration time between doses. These two examples with sulfur are well-known reducing agents that would be expected to react with chlorine. Other than that, most of the rest of the more reactive AAs have nucleophilic functional groups on their side chains with nitrogen and oxygen. Besides that it did not seem like any other factors changed the reactivity. Taking that into account the AAs will have different reactivities than those implied in the general mechanism generally cited in one form or another in the literature.

Implications for Amino Acid Chlorination Research

With the advancement of DBP formation detection, in chlorinated wastewater, reclaimed wastewater can begin to improve. In the past, studies have gone through with chlorination without knowing the extent of amino acid chlorination side reaction. Newfound advancement in understanding DBP formation, from amino acid chlorination, will also shed light on amino acid reactivity. If the initial products vary in type with AA, then the final DBPs present in treated wastewater will vary, and this may contribute to the several hundreds of DBPs speculated in the literature.

Is CE Viable for Detecting DBP Ions?

With CE we can view AA chlorination quickly, we will see DBP ions and from that we can determine how long the reaction lasts. CE is now another tool that can be used to accurately detect DBP ions that arise from chlorine disinfection. Now it is known that AAs do not react similarly, the mechanism will differ depending on the amino acid. From that future studies can take that into account while researching AA reactivities.

Limitations

Sources of Error

An overall caveat with using analytical methods to analyze AAs is that only one AA can be analyzed at a time, not as mixtures of different AAs which is what is seen in water. Studying them separately will yield an understanding of what they do individually, giving a picture of how reactivity changes in a mixture. CE as a method does introduce a degree of variability with each sample taken, over time if the capillary is changed for either a shorter or longer one, the migration time of whatever is being analyzed will be longer or shorter. Throughout my experiments, it was also noted that the migration time even between the same AAs will not be completely the same. The instrument itself has issues with its detection limit. If the compound's concentrations are too low, the instrument will not be able to detect what is inside the solution. Despite the potential sources of error, the advantages CE provides heavily outweigh it. With the advent of CE-

MS instrumentation, it is speculated that greater analytical power in terms of qualitative analysis of the DBP ions as well as lower detection limits will be realized in the future.

CHAPTER SIX

CONCLUSION

Recap of Key Findings

Study Significance

The interest lies in further delineating the mechanism(s) of amino acid chlorination and, further than that, what products arise are of interest. Also interested in looking at which steps of the mechanism will vary depending on the AA or chlorine ratio. Then see the outcome of that by determining if different DBPs are produced. Past research has found that in an amino acid chlorination reaction, harmful DBPs can be formed. In the past, during the chlorination of wastewater, DBPs that have amino acid precursors have yet to be well documented. This research can shed light on the mechanism and the products formed. What has been determined to be detected by CE are Amino acid ionic product peaks. The lower Cl_2:AA ratios investigated here most likely resulted in mono-chlorinated products, with that in mind we can say that they most likely included the N-monochloroamino acids initially. The electropherogram places most of these monochloroamine products in the negative zone, which also fits with the prediction of what the products are.

Using CE, the analysis will be qualitative with better product identification using mass spectrometry. CE-MS methods may prove superior due to the use of unreactive buffers and detection of ions. LC-MS methods can be used for the separation of neutral species; however, most LC methods rely on mobile phases

containing organic additives that in turn may react with the combined-chlorine products being studied. The reactivity of each AA with NaOCl should continue to be studied. As each AA is tested, the NaOCl concentration should be ramped to higher molar ratios to observe products at later steps in the mechanisms. The hypothesis is that as Cl concentration increases the amino acid peak will decrease and the number of products will increase based on the amino acid reactivity. From that a conclusion will be reached as to whether the reactions do indeed lead to DBPs like Monochloramines and Nitrogenous-DBPs, and what the reactivity is for our amino acids. The results here will aid in developing a new analytical method for assessing amino acid reactivity to chlorination, the method using CE as another tool.

APPENDIX A

GLOSSARY

ELECTROOSMOTIC FLOW - The sample solution moves due to the influence of a charged surface that occurs from an electric field.

ELECTROPHORETIC APPARENT MOBILITY - How the solute responds to an electrical field consequently the anions move towards the anode and the cations to the cathode.

MASS SPECTROMETRY - A tool that is used to measure the mass-to-charge ratio of samples,

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY - A method used to filter and separate neutral compounds in a solution.

PKA - This value determines how strong an acid is.

REDUCING AGENT - This is a species that gives an electron to a species that receives it. is a measure of how well the peak is being detected. The lower the rf the larger the uncertainty will be in detecting the peak from another peak. While the higher rf indicates there is little uncertainty meaning less noisy peaks.

RESPONSE FACTOR – Is a measure of how well the peak is being detected. The lower the rf the larger the uncertainty will be in detecting the peak from another peak. While the higher rf indicates there is little uncertainty meaning less noisy peaks.

REFERENCES

1. Ahern, K., & Rajagopal, I. (2012). Biochemistry Free & Easy, 43. (https://oregonstate.app.box.com/s/i5zdnuolgda8snovflueqazdv4q4ikiw)
2. Bond, T., Templeton, M. R., & Graham, N. (2012). Precursors of nitrogenous disinfection by-products in drinking water—A critical review and analysis. Journal of Hazardous Materials, 235-236, 1-16. https://doi.org/10.1016/j.jhazmat.2012.07.017
3. Bond, Jin Huang, Michael R. Templeton, Nigel Graham, Occurrence and control of nitrogenous disinfection by-products in drinking water – A review, Water Research, Volume 45, Issue 15, 2011, Pages 4341-4354, ISSN 0043-1354, https://doi.org/10.1016/j.watres.2011.05.034. (https://www.sciencedirect.com/science/article/pii/S0043135411003071)
4. Cai, L., Li, L., Yu, S., Guo, J., Kuppers, S., & Dong, L. (2019). Formation of odorous by-products during chlorination of major amino acids in East Taihu Lake: Impacts of UV, UV/PS and UV/H2O2 pre-treatments. Water Research, 162, 427-436. https://doi.org/10.1016/j.watres.2019.07.010
5. Hong, H. C.; Wong, M. H.; Liang, Y. Amino Acids as Precursors of Trihalomethane and Haloacetic Acid Formation during Chlorination. Archives of Environmental Contamination and Toxicology 2008, 56 (4), 638–645.

https://link.springer.com/article/10.1007/s00244-008-9216-4

6. How, Z. T.; Linge, K. L.; Busetti, F.; Joll, C. A.

   Chlorination of Amino Acids: Reaction Pathways and Reaction Rates

   Environmental Science & Technology 2017 51 (9), 4870-4876

   DOI: 10.1021/acs.est.6b04440

7. Hureiki L., J.P. Croué, B. Legube, Chlorination studies of free and combined amino acids, Water Research, Volume 28, Issue 12, 1994, Pages 2521-2531, ISSN 0043-1354, https://doi.org/10.1016/0043-1354(94)90070-1.-

   (https://www.sciencedirect.com/science/article/pii/0043135494900701)

8. Kanchanamayoon, W. Sample Preparation Methods for the Determination of Chlorination Disinfection Byproducts in Water Samples. Chromatographia 78, 1135–1142 (2015). https://doi.org/10.1007/s10337-015-2934-7

   (https://www.sciencedirect.com/science/article/pii/S0021967398007523)

9. Kozari, A.; Paloglou, A.; Voutsa, D. Formation Potential of Emerging Disinfection By-Products during Ozonation and Chlorination of Sewage Effluents. Science of The Total Environment 2020, 700, 134449

10. Lazarova, V., Savoye, P., Janex, M., Blatchley, E., & Pommepuy, M. (1999). Advanced wastewater disinfection technologies: State of the art and perspectives. Water Science and Technology, 40(4-5), 203-213. https://doi.org/10.1016/S0273-1223(99)00502-8
11. S.F.Y. Li, Y.S. Wu, ELECTROPHORESIS | Capillary Electrophoresis, Editor(s): Ian D. Wilson, Encyclopedia of Separation Science, Academic Press, 2000, Pages 1176-1187, ISBN 9780122267703, https://doi.org/10.1016/B0-12-226770-2/04321-0. Year=2000
12. Li, T., Wang, Z., Wang, C., Huang, J., & Zhou, M. (2022). Chlorination in the pandemic times: The current state of the art for monitoring chlorine residual in water and chlorine exposure in air. Science of The Total Environment, 838, 156193. https://doi.org/10.1016/j.scitotenv.2022.15619
13. Manaenkov, O. V., Sidorov, A. I. & Sul'man, E. M. Rapid Determination of Amino Acids by Capillary Electrophoresis without Preliminary Derivatization. J. Anal. Chem. 58, 979–982 (2003)
14. Mishra, B. K., Gupta, S. K. & Sinha, A. Human health risk analysis from disinfection by-products (DBPs) in drinking and bathing water of some Indian cities. J Environ Health Sci Eng 12, 73 (2014)
15. Na, C.; Olson, T.M. Mechanism and Kinetics of Cyanogen Chloride Formation from the Chlorination of Glycine. Environ. Sci. Technol. 2006, 40, 1469-1477.

16. Stanley B. Analysis of Amino Acid Precursors and Reaction Products from the Disinfection of Water and Wastewater, 2022
17. Ta, H. Y., Collin, F., Perquis, L., Poinsot, V., Ong-Meang, V., & Couderc, F. (2021). Twenty years of amino acid determination using capillary electrophoresis: A review. Analytica Chimica Acta, 1174, 338233. https://doi.org/10.1016/j.aca.2021.33823
18. Trehy, M. L.; Yost, R. A.; Miles, C. J. Chlorination Byproducts of Amino Acids in
19. Natural Waters. Environmental Science & Technology 1986, 20 (11), 1117–1122.
20. Biochemistry Free For All. Structure and Function - Amino Acids. {https://bio.libretexts.org/Bookshelves/Biochemistry/Book%3A_Biochemistry_Free_For_All_%28Ahern_Rajagopal_and_Tan%29/02%3A_Structure_and_Function/202%3A_Structure__Function_-_Amino_Acids. 2015, Pg. 55.
21. U.S. Environmental Protection Agency. Wastewater Technology Fact Sheet Chlorine Disinfection. 1999

https://www3.epa.gov/npdes/pubs/chlo.pdf.