SYNTHESIZING AND CHARACTERIZATION OF AMINO ACID BASED IONIC LIQUIDS FOR PHARMACEUTICAL APPLICATIONS

ARASH YAZDANI

CHEMICAL ENGINEERING

UNIVERSITI TEKNOLOGI PETRONAS

SEPTEMBER 2015

Synthesizing and Characterization of Amino Acid Based Ionic Liquids for Pharmaceutical Applications

by

Arash Yazdani

17043

Dissertation submitted in partial fulfilment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

SEPTEMBER 2015

Universiti Teknologi PETRONAS,
32610, Bandar Seri Iskandar,
Perak Darul Ridzuan.

CERTIFICATION OF APPROVAL

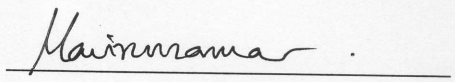
Synthesizing and Characterization of Amino Acid Based Ionic Liquids for Pharmaceutical Applications

by

Arash Yazdani
17043

A project dissertation submitted to the
Chemical Engineering Programme
Universiti Teknologi PETRONAS
in partial fulfilment of the requirement for the
BACHELOR OF ENGINEERING (Hons)
(CHEMICAL ENGINEERING)

Approved by,

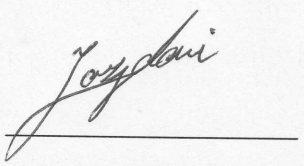


(Dr. Muhammad Moniruzzaman)

UNIVERSITI TEKNOLOGI PETRONAS
BANDAR SERI ISKANDAR, PERAK
September 2015

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgments, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.



ARASH YAZDANI

ABSTRACT

There has been a growing attention towards amino acid based ionic liquids (AAILs) since the past decade. Synthesizing ionic liquids (ILs) from renewable biomaterials is a promising method to improve the biocompatibility and biodegradability of ionic liquids. In this project, 10 choline based ILs were synthesized with different amino acids and tested towards gram-positive and gram-negative bacteria to show the relationship between the anion structure and the degree of toxicity. All the AAILs demonstrated a high biocompatibility towards the tested bacteria and their toxicity decreased by smaller molecular size of anion. Additionally, biodegradability of the synthesized AAILs were assessed via the method of closed bottle test employing river water microorganisms and it turned out that all the AAILs had over 60% biodegradation which classifies them as “readily biodegradable”. Longer chain length and presence of functional groups in the structure of anion directly affected the molecular breakdown by microbial activities.

ACKNOWLEDGMENT

Firstly I would like to raise my appreciation to Universiti Teknologi PETRONAS (UTP) and Center Of Research in Ionic Liquids (CORIL) for providing the facilities.

Next, I would like to express my kindest gratitude to my supervisor Dr. Moniruzzaman for his guidance and continuous support through the success of my bachelor thesis.

Besides my advisor, I am so grateful to thank Dr. Magaret Sivapragasam and the Ionic Liquids team for providing an absolute care and insightful comments.

Last but not least, I place on record, my sense of heartfelt gratitude to my family and my friends, especially my beloved soulmate for the consistent emotional encouragement and love.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AA	Amino Acids
AAIL	Amino Acid Based Ionic Liquids
BOD	Biological Oxygen Demand
DI	Deionized
EC	Effective Concentration
FYP	Final Year Project
H-NMR	Proton- Nuclear Magnetic Resonance
IL	Ionic Liquid
MHB	Muller-Hinton Broth
NMR	Nuclear Magnetic Resonance
OECD	Organization for Economic Cooperation and Development,
ppm	Parts Per Million
rt	Room Temperature
RTILs	Room Temperature Ionic Liquids
UTP	Universiti Teknologi PETRONAS

LIST OF APPENDICES

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Ionic liquids (ILs) are referred as liquid salts, comprising of anions and cations. They are not a new discovery but their importance had not been recognized any earlier than the past few decades. Since then, there has been a growing attention towards this field and its applications due to the unique properties of ILs. Impressive physicochemical properties such as low boiling point, low vapor pressure, thermal and chemical stability, electrical conductivity as well as a number of advantages over conventional organic solvents in solubility fields has widen the application of ILs to many aspect of chemical engineering, nanotechnology, biotechnology and pharmaceutical (Lucia et al., 2012 ; Tushar et al., 2011). It is not possible to expect all salts to be in liquid form in ambient temperature (20 -26°C). This is because of the strong ionic bonds between cations and anions in a well-arranged crystalline structure. However, ILs can be synthesized in a way to be liquid at normal temperature by selecting their cations and anions bulky and asymmetrical (Balk, Holzgrabe, & Meinel, 2015).

Applications of ILs in industrial sectors are still in an infant stage. Albeit many studies and researches have been carried out. In order to take advantage of these potential chemical compounds in industries, there should be a comprehensive understanding about their physical and chemical properties and the use of right ILs for a specific industrial application.

Introducing new applications of ILs in large scales requires considerations not only about the process but the potential hazards to the environment. Chemical and pharmaceutical plants are of major sources of pollutions, therefore they are responsible for providing alternatives which reduce the environmental hazards and health risks. Furthermore, many publications have claimed ILs as green solution to industry besides their potential applications in many fields. In contrast there should be more researches highlighting the concerns with toxicity and the methods of recycling in the environment (Hu et al., 2007; Tang et al., 2010).

With respect to wide variety of cation and anion molecules, it is possible to synthesize over 10^{14} unique ILs with different physical and chemical properties (Davis, Hada, Herring III, & Eden, 2014). Ionic liquids can be characterized by their degree of biocompatibility and biodegradability, a measure which reveals the toxicity or environmental friendliness profile of the ILs. It is very important for ILs to be readily biodegradable in case of their release to aquatic environment. On the other hand, the increasing number of applications in pharmaceutical industries puts more emphasize on biocompatible ILs.

The role of amino acids in synthesizing ILs can be found by recognizing their biodegradability and bioactivity features. Since the amino acid molecules are consisted of two functional groups of carboxylic acid and an amino group, they can be used as cation or anion in forming IL molecules in a variety of kinds. This variety of kind in synthesizing ILs from amino acids enables us to find a better link their characterizations to their molecular structure as we can analyze more types of ILs and hopefully to a pattern of design which could be used to synthesize them for object-oriented applications.

Biocompatibility and biodegradability are of characteristics of ILs which tie them up with their practically in actual chemical and pharmaceutical process. An ideal IL can be defined by possessing both of these characteristics while saving their unique properties. However it is impossible to gather all the properties in one IL, therefore, it would be more practical if we design certain ILs for task-specified applications.

1.2 Problem Statement

There is comparatively less information and data analysis than the possible types of synthesized ILs, especially those related to fine-tuning of them. Many different types of ILs are being synthesized every day but their practicality and sustainability remains unanswered without recognizing their properties and characteristics. With so many synthesizable possibilities, it seems intensive to synthesize and test the properties of all possibilities to find their applications. What is more, ILs have provided a great field of applications for pharmaceutical industries and other biological applications, but this requires an acceptable degree of biocompatibility and biodegradability of ILs. On the other hand, biodegradability of the ILs also has to be proven before industrial implications, this is due to their crucial toxicity impacts and environmental concerns which recently have been reported (Ventura et al., 2012; Gouveia et al., 2014).

1.3 Objectives

It is desired to prepare a list of biocompatibility and biodegradability profile of ILs according to their cation and anion molecules and the alkyl side chain length involved in their structure, however due to the vast range of possible ionic molecules, it will be only focused on the Amino Acid based ionic liquids with cholinium [Cho] cation and with various anion molecule ([Cho][AA]).

There are two preliminary aims followed in this project as titled below:

1. To synthesize and analyze the biocompatibility & biodegradability of AAILs using an eco-toxicity test.
2. To demonstrate the relationship between the different amino acid anions and biodegradability & biocompatibility profile of the AAILs.

1.4 Scope of Study

The method of synthesizing ILs based on amino acids has dissolved the issue with biodegradability to some extent, however many factors still affect toxicity such as types of cations, anions and length of alkyl side chain. In this project, it is objected to discover the role of different amino acid anions in the degree of biocompatibility and biodegradability. The role of the anion is of high importance to identify as the biomaterial derived cations have exceptional permeability to cells structure (Egorova, Seitkalieva, Posvyatenko, & Ananikov, 2015).

Some specific microorganisms will be used to determine the biocompatibility of the AAILs and to analyze the possible effects of AAILs on the microbes. And also analyzing biodegradability profile of AAILs in possible natural water sources such as lakes, in the case of industrial waste release.

It is found that it is not possible to have all of the desired properties in one type of ILs, hence it is aimed to compromise between the desired properties of AAILs in order to be suitable for pharmaceutical uses which makes it possible to find a general method for synthesizing and fine-tuning task specific ILs.

CHAPTER 2

LITERATURE REVIEW

2.1 Amino Acid based Ionic Liquids

In the last decade, several ionic liquids had been synthesized and characterized in researches (first generation). Later, due to highly desired properties of ILs, some task specific ILs were synthesized for industry applications (second generation). Another class of ILs has been recently developed to suit pharmaceutical applications based on the anion and cation combinations (third generation) (Costa et al., 2015).

It has been suggested by Lopes et al. (2013) to use biomolecules cations and anions to synthesize ILs which are more biodegradable and have lower toxicity. The examples of this method are such as natural amino acid, choline, fructose and lactic acid bases to synthesize ILs. Among these, amino acids have more than a few advantages in synthesizing ILs, the first advantage is that they have both a carboxylic group and an amino group and carboxylic acid residue in their molecule which can act as cation and anion, with various side chains and chiral carbon atom. Amino acid ionic liquid (AAIL) are expected to show a better biodegradability and biocompatibility compared to other types of ILs due to the presence of amino acids in natural living species. The other advantage amino acids are available at low costs and can be obtained in large quantities and high purities, as stated by Ohno and Fukumoto (2007).

2.2 Biocompatibility and Biodegradability

As it is stated by Black (2005) the biocompatibility is referred to the quality of interaction of between living organs or tissues and non-living materials. This is described as the effect that material leave on the living tissues. It is highly desired to extend this quality between the ILs and living organs as it widens their applications in this field even more.

Biodegradation as another important factor for ILs to be recognized as environmental friendly. The biodegradation degree shows how readily a compound goes through chemical dissolution by biological means such as microbes. ILs by having a negligible vapor pressure, prevent from air pollution but their release to environment without any knowledge about toxicity and measured considerations could have lethal effects to environment and aquatic lives. What is more, some ILs, because of their high stability towards abiotic and biotic degradation processes can be persistent pollutants in soil and water (Markiewicz et al., 2013).

2.3 Toxicity Assessment

Determining toxicity of the ionic liquids is a common mode in order to estimate their environmental and health impact. Many researchers suggest that biodegradability and biocompatibility characteristics of AAILs change based on their cation, anion and alkyl side chain length used in the structure of ILs. Many literatures have discussed the role of these factors in the biodegradability and toxicity behavior of ILs and in plenty of cases some similarities have been found. The experiments done by Markiewicz et al. (2013) reveal that the head groups and cations were found to have no significant effect on the toxicity, therefore the toxicity of the ILs can only be attributed to the anion.

The analysis of experiments show that among different anions, the toxicity increases as the solubility decreases. The trend of toxicity by anions can be presented in

this order: $N(CN)_2^- < B(CN)_4^- \sim (CF_3SO_2)_2N^- < (C_2F_5)_3PF_3$. This statement, however is opposed by Lopes et al. (2013) which mentions that anion exerts only slight changes in the toxicity behavior of the molecule. The toxicity of ILs escalates as the alkyl side chain length increases. This is also proven by almost all the studies including a research done by Dochetry, Dixon and Kulpa Jr., (2007) and later by Romero, Santos, Tojo, and Rodriquez (2007) and also Lopes et al. (2013).

The role of anion is highly important since in some cases of amino acid derived ionic liquids it can have adverse effects. An unexpected outcome of a cytotoxicology experiment shows that the toxicity is even more in amino acid based ILs more than many other ILs (Egorova et al., 2015). These results are justified by expressing the ability of amino acids to enter the cells through special membrane transporter proteins. It was suggested that the amino acid cations enhance the permeability of potentially toxic anions such as tetrafluoroborate (BF4) to the cell, therefore, results in a high toxicity, signifying governing effect of the anion. However the role anion can be seen in these results where they leave different effects on the same bacteria but in overall, they demonstrated harmless behavior.

Pirydinium and imidazolium as common head groups in the structure of ILs demonstrate different degree of toxicity. A set of experimental data revealed by Gouveia et al. (2014) shows that the imidazolium based ILs were slightly more toxic to crustacean *Artemia salina* (an invertebrate organism) than pyridinium. Similarly, in both head groups, the toxicity increased with increasing the length of the alkyl chain for the same bromide anion. The same effects are expected on other types of marine organisms. Changing the bromide anion to an amino acid carboxylate for the same cation showed no effect on IL toxicity. However, when both the anion and cation were obtained from biomaterials such as choline [Cho] and amino acids [AA] there was a big difference found in such that the toxicity decreased by two orders of magnitude (hundred times lower).

Toxicity of ILs on soil bacteria, as it is presented in the research performed by Gouveia et al. (2014) shows that different types of bacteria show different reactions to

the same ILs tested. Between the two types of bacteria, *B. subtilis* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria), the *E. coli* bacteria was less sensitive to many ILs in comparison with gram positive bacteria. This is reported as different interaction between the ILs and gram positive bacteria's peptidoglycan and lipid components of the cell wall. Therefore it is necessary to test chemical compounds on different types of living organisms and microorganisms as there are different interactions between biological cells and chemical compounds.

There are different classifications to toxicity test results according to exposure time (acute or chronic), mode of effect (death, growth, reproduction), or effective response (lethal, sublethal) (Neumegen, Fernandez-Alba, & Chisti, 2004). In order to perform and demonstrate the results of the toxicity tests, methods of Minimum Inhibitory Concentration (MIC) and Effective Concentration (EC_{50}) are commonly used. EC_{50} is the concentration of a chemical substance for a given exposure time which reduces the biological activity by half, which is categorized as a measure of acute toxicity. Biological activity can be taken as any biological characteristic of a living species such as reproduction, cell growth, or biodegradation. MIC on the other hand, as a measure of effective response is used to find the minimum concentration of chemical substance that inhibits a biological activity sensibly. Impressive biological activity of AAILs and the possibility of tailoring for cell targeting creates a wide range of applications in anticancer drugs, modulation of virulence of pathogens in obtaining vaccinal strains, modification of tumor and cancer immunotherapy (Egorova, et al., 2015).

According to Vineet and Sanjay (2010), about 50% of the drugs are administrated as salts. Considerable amount of physicochemical and biopharmaceutical properties of pharmaceuticals can be paired by a basic or acidic drug agent with an opposite charge ion to create salt mode of drug. These salts may provide appreciable advantages in terms of solubility, dissolution, thermal stability, crystallinity and etc. With these characteristics they could have enormous influence on the quality, safety and performance of the drugs, which could assist formulators in numerous contributions of drug discovery and development. And their pharmacodynamics and toxicological effects on nature and environment may undergo evaluation and modification.

CHAPTER 3

METHODOLOGY

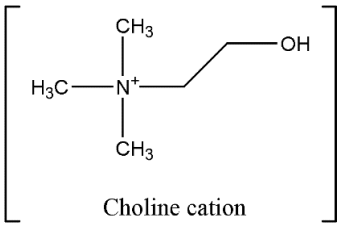
3.1 Synthesize of Amino Acid Based Ionic Liquids

In this project different amino acid based ionic liquids (AAILs) were synthesized to analyze their toxicity and biodegradability.

ILs can be synthesized via many types of natural products through relative green methods like ion exchange and neutralization methods. Depending on the type of the ILs synthesized, different compounds and solvents are required. In this project the cation part of the ILs will be cholinium based and the anion part varies based on amino acids. Three main steps are proposed by Kagimoto, Taguchi, Fukumoto, and Ohno (2010) which could be used to synthesize a number of amino acid based ILs. These three steps are:

1. Quaternization: quaternizing a compound through reaction in order to provide the cation of ILs. In this case the cation is cholinium.
2. Anion Exchange: exchanging the anion between the initial quaternized compound and a solvent (usually water/methanol).
3. Neutralization with Amino Acid: addition of an aqueous amino acid solution to the aqueous quaternized anion exchanged solution. Then this mixture is evaporated and unreacted amino acid can be filtered by adding a mixture to the solution which amino acid does not dissolve by.

Cation



Anion Derived Molecules

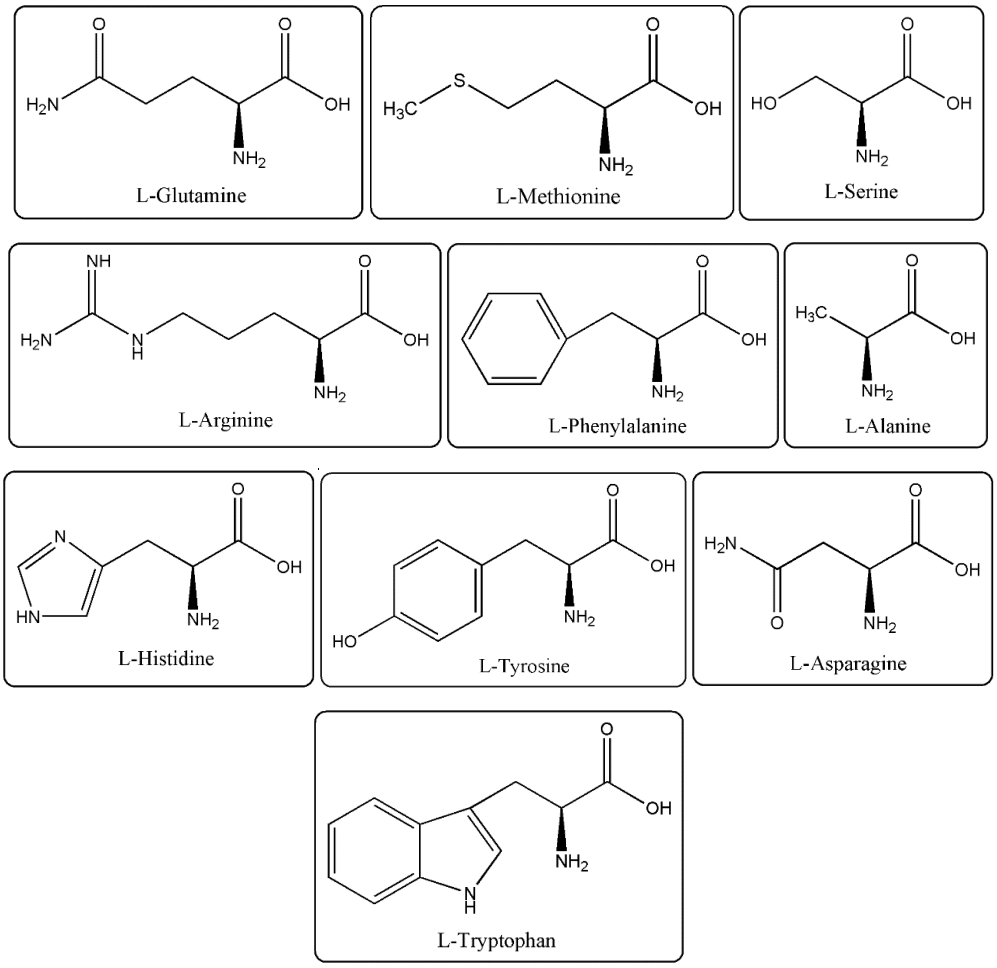


FIGURE 3.1 Molecular structure of cation and amino acids used in synthesize of AAILs.

Different amino acids were selected with various functional groups and side chains to react with choline hydroxide solution in order to synthesize amino acid based ionic liquids. The molecular structure of these amino acids and choline-cation ([Cho]) is illustrated in Figure 3.1.

3.2 Microorganisms Growth and Culture Conditions

Bacteria are sharing similar cellular structure and reproduction mechanism. The reproduction occurs by providing the condition of growth and then the cells start to grow in size and by the time which the components of new cell, e.g., protein, RNA, etc., is doubled, cell division initiates (Posten & Cooney, 1993).

Microorganisms need water and a nutrition source to grow and it is desired to control the moisture in the nutrition source. Bacteria can be cultured rapidly through the following steps:

1. Preparing culture dishes (petri) and agar solution.
2. Dispersing a dilute suspension of cells.
3. Providing condition of growth (Temperature, Moisture and Nutrients).
4. Controlling the population by time.

In concern with growth of bacteria, temperature and time are critical factors to control the growth. The temperature adversely affects the time of growth and reproduction of bacteria. Nutrition sources are needed for metabolic functions of bacteria, and are widely available.

3.3 Biocompatibility and Biodegradability Analysis

The effect of amino acid derived ILs on natural microorganisms is assessed via eco-toxicity tests and the effect of biodegradables on the AAILs in terms of biodegradation is assessed via closed bottle test.

3.3.1 Biocompatibility Test

There are many types of bacteria to conduct toxicity tests on, they are varied by their ecosystem (aquatic and soil bacteria) and also by biological features (vertebrate and invertebrate). The toxicity tests will be done on four types of bacteria, namely:

- *Escherichia coli*
- *Aeromonas hydrophila*
- *Listeria monocytogenes*
- *Staphylococcus aureus*

Escherichia coli and *Aeromonas hydrophila* are gram negative bacteria which are commonly found in the lower intestine of warm blooded organisms and water respectively. *Listeria monocytogenes* and *Staphylococcus* aureus are of gram positive bacteria and found in soil plus water and in human respiratory tract respectively.

These bacteria will come in direct contact with each of five different AAILs ([Cho][AA]) with the same cation (cholinium) with different concentrations and then the method of effective concentration (EC_{50}) will be employed here to determine the concentration of toxicants which causes growth of bacteria to reduce by 50%. These steps were repeated to obtain 2 replicated set of data out of independent experiments.

In this project Muller-Hinton Broth (MHB) at concentration of 21g/L was prepared as a source of minerals and vitamins necessary for bacterial growth and as a

medium to bring the bacteria in contact with AAILs. This medium after being inoculated with each of the bacteria was placed in a shaker with a controlled temperature of 37˚C and rotation speed of 175 rpm. The initial density of the bacteria was measured as a fraction of radiation adsorption by microplate reader. This technique was used again to determine the density of bacteria 24 hours after being incubated with ionic liquid to find out viability (%) of the bacteria per concentration in a 96-well plate. By plotting viability (%) versus concentration it is possible to estimate the concentration in which the viability is reduced by half (EC_{50}).

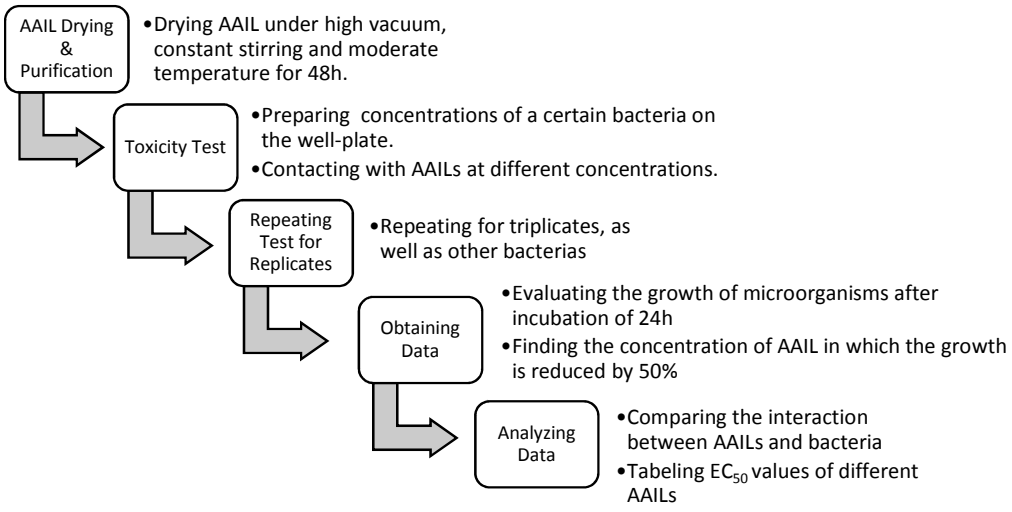


FIGURE 3.2 Biocompatibility Assessment of AAILs

3.3.2 Biodegradability Test

In order to analyze the resistance of the AAILs in the case of industrial or accidental release to the environment, biodegradability test will be performed. Samples were taken from the most possible destination of these industrial wastes which are sewage water, rivers and lakes at many locations. By performing Biological oxygen demand (BOD) experiment on the AAILs, it is possible to predict and compare the biodegradability of different AAILs in natural water resources.

BOD test as described in OECD 301 (Closed bottle Test) method of experiment suggests that the biodegradability is assessed by calculating the percentage oxygen uptake of the theoretical oxygen uptake (TheOD) after 28 days. (Organization for Economic Cooperation and Development, OECD, 2001).

$$BOD = \frac{\text{mg } O_2 \text{ uptaken by test substance} - \text{mg } O_2 \text{ uptake by blank}}{mg\ test\ substance\ in\ vessel} \quad (1)$$

The biodegradation then can be obtained by:

$$\%\ biodegradability = \frac{BOD\ (mg\ O_2\ /mg\ substance)}{ThOD\ (mg\ O_2\ /\ mg\ substance} \ X\ 100\% \quad (2)$$

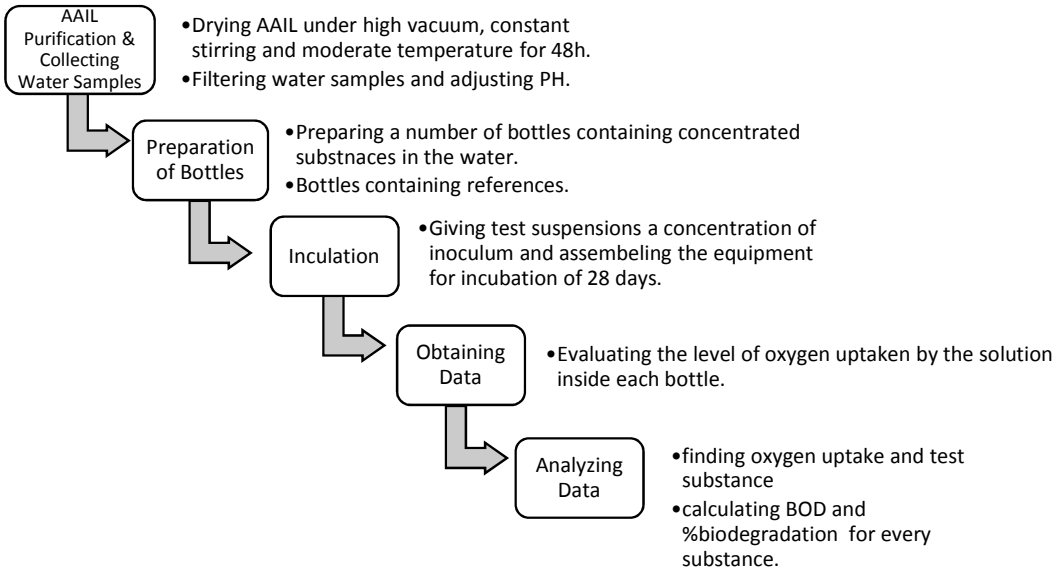


FIGURE 3.3 Biodegradability Assessment of AAILs

According to the closed bottle test procedure a concentration of four minerals was prepared and added to dilution water as follows:

- Potassium dihydrogen orthophosphate, KH_2PO …….. 8.5 mg/mL
- Dipotassium hydrogen orthophosphate, K_2HPO_4 …….. 21.7 mg/mL
- Disodium hydrogen orthophosphate dihydrate, $Na_2HPO_4.2H_2O$ ……..… 33.4 mg/mL
- Ammonium chloride, NH_4Cl …….. 0.5 mg/mL

Dissolved in 20mL DI water.

- Calcium chloride, anhydrous, $CaCl_2$ …….. 27.5 mg/mL

Dissolved in 10mL DI water.

- Magnesium sulphate heptahydrate, $MgS0_4.7H_20$…….. 22.5 mg/mL

Dissolved in 10mL DI water.

- Iron (III) chloride hexahydrate, $FeCl_3.6H_20$ …….....…….. 4.1 mg/mL

Dissolved in 20mL DI water.

1 mL of each of the solutions was added to the dilution water, aerated for 1 hour at the test temperature (22˚C) and left for about 3 hours to have a dissolved oxygen concentration at equilibrium in the mentioned temperature.

According to OECD 301, the concentration of test substance which in this case is the synthesized ionic liquids, should be 1-10 g/l based on solubility. It was tried to keep this concentration about 2 g/l however the exact weight of the ionic liquids are recorded for the purpose of biodegradability calculation, as per attached in Appendices C.

A necessary part of the biodegradability calculation, is the theoretical oxygen demand. This should be calculated for all the test substances to compare the oxygen uptake in the experiment condition compared to the oxygen uptake assuming total degradation of the substance. The method described by Gavhane (2012), was used to calculate the theoretical oxygen demand of ionic liquids as tabulated below:

Table 3.1 Theoretical oxygen demand of synthesized AAILs

Ionic Liquid	TheOD (mg_{O2}/mg substance)	Ionic Liquid	TheOD (mg_{O2}/mg substance)
[Cho][Ala]	1.664	**[Cho][Met]**	1.839
[Cho][Arg]	1.442	**[Cho][Phe]**	2.027
[Cho][Asp]	1.360	**[Cho][Ser]**	1.460
[Cho][Glu]	1.476	**[Cho][Trp]**	1.926
[Cho][His]	1.487	**[Cho][Tyr]**	1.857

The oxygen content of the bottles were measured by OxiTop equipment after incubation daily to track the level of oxygen uptake as a result of biodegradation. The difference between the levels of oxygen measurement is taken as BOD.

3.4 Milestone

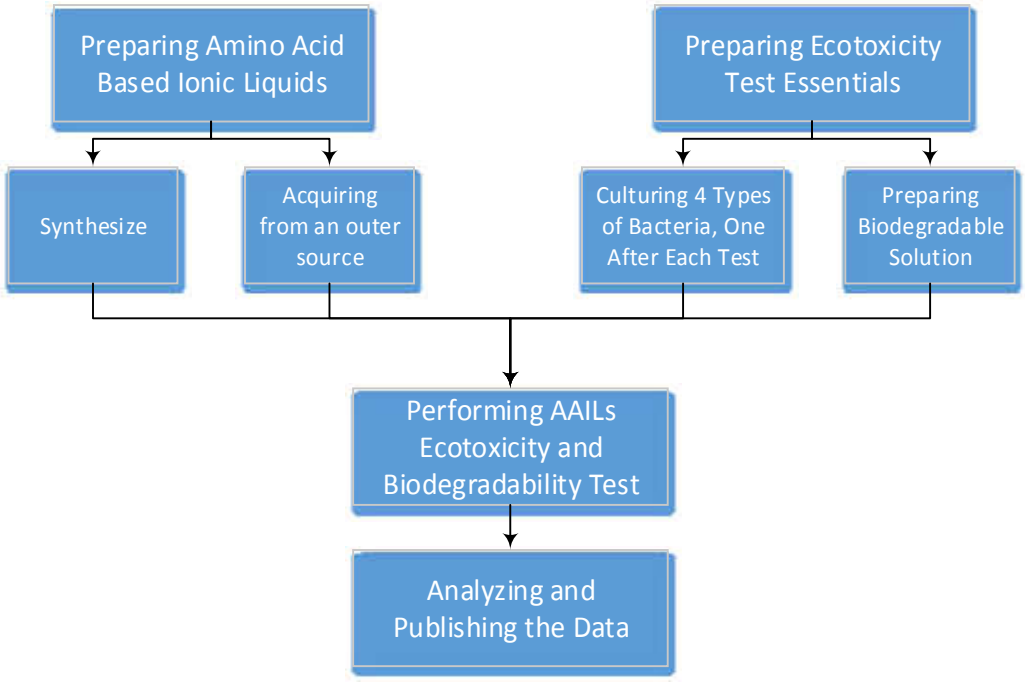
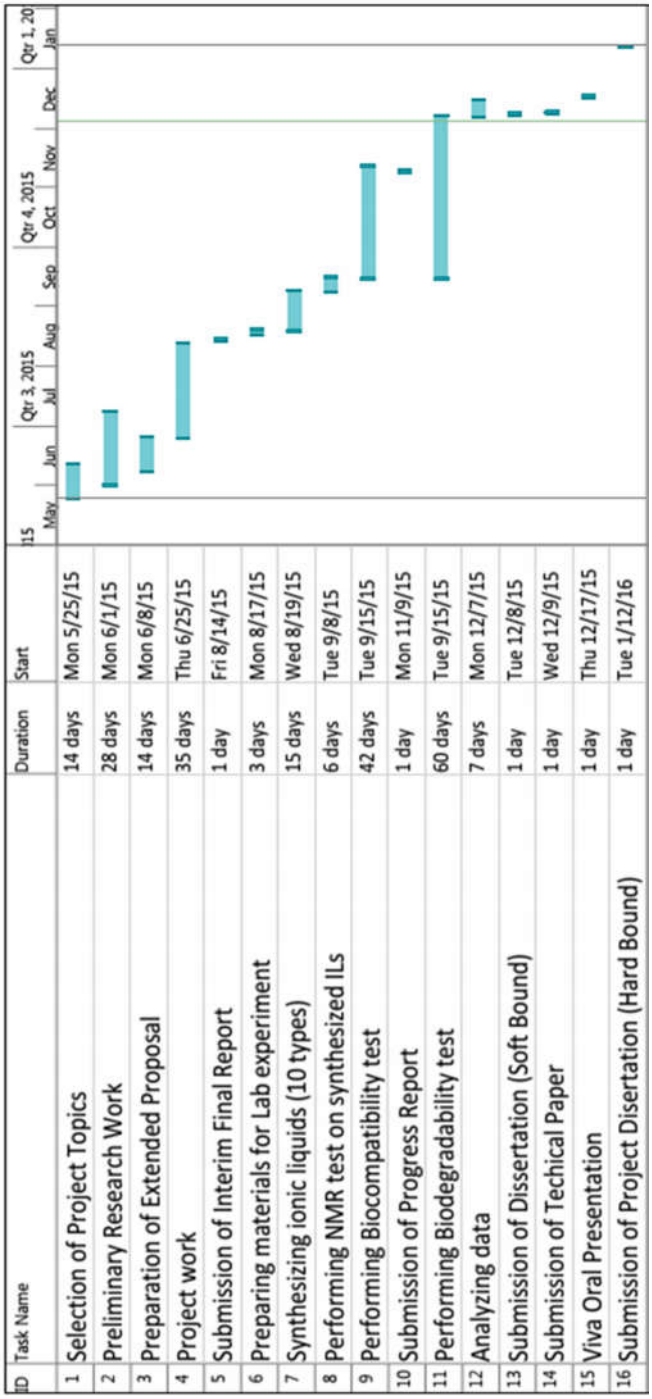


FIGURE 3.4 Project overview and milestone

3.5 Gantt Chart



ID	Task Name	Duration	Start
1	Selection of Project Topics	14 days	Mon 5/25/15
2	Preliminary Research Work	28 days	Mon 6/1/15
3	Preparation of Extended Proposal	14 days	Mon 6/8/15
4	Project work	35 days	Thu 6/25/15
5	Submission of Interim Final Report	1 day	Fri 8/14/15
6	Preparing materials for Lab experiment	3 days	Mon 8/17/15
7	Synthesizing ionic liquids (10 types)	15 days	Wed 8/19/15
8	Performing NMR test on synthesized ILs	6 days	Tue 9/8/15
9	Performing Biocompatibility test	42 days	Tue 9/15/15
10	Submission of Progress Report	1 day	Mon 11/9/15
11	Performing Biodegradability test	60 days	Tue 9/15/15
12	Analyzing data	7 days	Mon 12/7/15
13	Submission of Dissertation (Soft Bound)	1 day	Tue 12/8/15
14	Submission of Techical Paper	1 day	Wed 12/9/15
15	Viva Oral Presentation	1 day	Thu 12/17/15
16	Submission of Project Disertation (Hard Bound)	1 day	Tue 1/12/16

FIGURE 3.5 Gantt chart (FYP I & II)

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Synthesize of Amino Acid Based Ionic Liquids

Choline based amino acid ionic liquids were synthesized by the method of neutralization of acid and base and as a result of this reaction salt and water is produced.

The choline component of the ionic liquids was obtained from choline hydroxide 45% in methanol and reacted in 15% excess with amino acid dissolved in methanol/ethanol, for two days. The solvents and produced water separated using rotary evaporator. 10 types of choline based ionic liquids were synthesized by using 10 amino acids, namely are L-Alanine, L-Arginine, L-Asparagine, L-Glutamine, L-Histidine, L-Methionine, L-Phenylalanine, L-Serine, L-Tryptophan and L-Tyrosine. The physical state, molecular weight and the yield of these ionic liquids are presented in Table 4.1.

Table 4.1 Physical properties and yields of synthesized ionic liquids

Ionic Liquid	Physical state at rt*	Molecular weight ($g.mol^{-1}$)	Yield (%)
[Cho][Ala]	Liquid	192.26	96
[Cho][Arg]	Liquid	277.36	35
[Cho][Asp]	Liquid	235.28	88
[Cho][Glu]	Liquid	249.31	94
[Cho][His]	Liquid	258.32	81
[Cho][Met]	Liquid	252.37	96
[Cho][Phe]	Liquid	268.35	95
[Cho][Ser]	Liquid	208.26	86
[Cho][Trp]	Liquid	307.39	79
[Cho][Tyr]	Liquid	284.35	51

*rt: Room Temperature (25˚C)

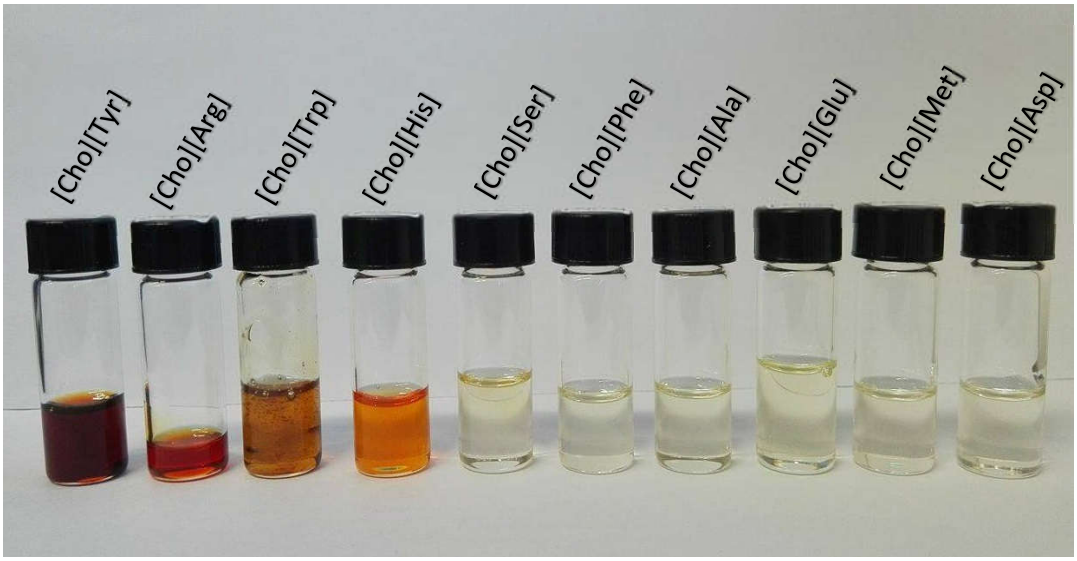


FIGURE 4.1 Image of synthesized AAILs

Based on the observations during synthesizing these AAILs, higher the solubility of the amino acids in the solvent provides a higher yield of reaction. In the cases where

the yield is not satisfactory, the solubility of the amino acid in methanol/ethanol was limited, therefore less amount of amino acid is reacted and less salt is produced. A good replacement of methanol/ethanol as solvent could be water, while noticing that, removing water is not as easy as removing methanol/ethanol from the product mixture.

H-NMR analysis has been performed to verify the purity of synthesized ionic liquids. As an example, the following NMR spectrum which belongs to [Cho][Trp] shows that the neutralization reaction is complete and the sample is as the name defines. H-NMR spectrum of the other synthesized AAILs is contained in Appendices A.

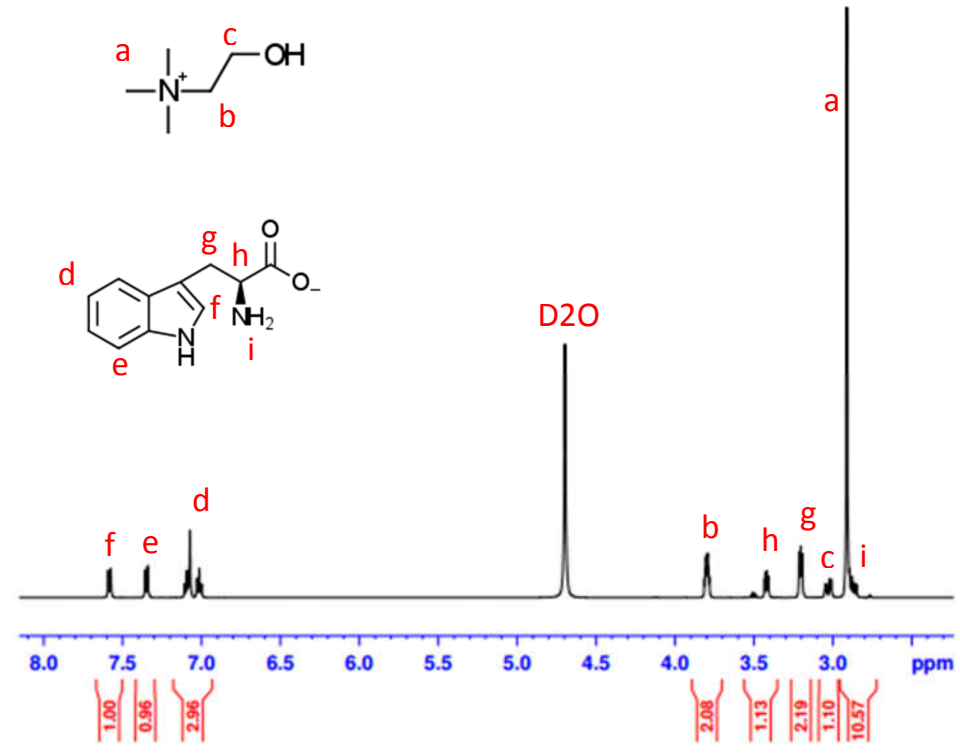


FIGURE 4.2 H-NMR spectrum of [Cho][Trp]

4.2 Biocompatibility Test

These 10 ionic liquids were synthesized to test their effect on the different types of bacteria. A series of ionic liquids' concentration from 10000 mg/L to 5 mg/L were prepared and incubated with bacteria for a period of 24 hours. The effective concentration as well as the upper and lower limit of analyzed data are illustrated in the table below:

Table 4.2 Effective concentration of synthesized ionic liquids to different bacteria

Ionic Liquid	***Escherichia coli*** **24h EC_{50} ($mg.L^{-1}$) (low-high)**	***Listeria monocytogenes*** **24h EC_{50} ($mg.L^{-1}$) (low-high)**	***Staphylococcus aureus*** **24h EC_{50} ($mg.L^{-1}$) (low-high)**	***Aeromonas hydrophila*** **24h EC_{50} ($mg.L^{-1}$) (low-high)**
[Cho][Ala]	1874 (1563-2246)	1295 (780-2151)	547 (218-1375)	839 (557-1265)
[Cho][Arg]	616 (364-1042)	767 (402-1466)	212 (167-268)	298 (220-403)
[Cho][Asp]	1052 (605-1828)	1057 (590 – 1894)	940 (446-1979)	1133 (713-1800)
[Cho][Glu]	934 (640-1362)	835 (575-1211)	231 (171-310)	1069 (772-1480)
[Cho][His]	1275 (950-1969)	605 (382-945)	633 (323-1242)	393 (213-726)
[Cho][Met]	709 (424-1186)	1580 (1074-2324)	609 (371-998)	554 (343-894)
[Cho][Phe]	5664 (4726-6788)	630 (491-805)	251 (194-326)	894 (594-1344)
[Cho][Ser]	1148 (876-1990)	1177 (718-1929)	716 (554-925)	1062 (736-1532)
[Cho][Trp]	627 (450-874)	440 (353-548)	203 (141-291)	640 (373-1100)
[Cho][Tyr]	2223 (1670-2960)	364 (196-675)	323 (225 – 464)	348 (221-549)

The respective dose response curve for every test can be found in Appendices B.

It was expected by using biomaterial (amino acid) derived cations and anions, biodegradability and biocompatibility of ionic liquids to be improved. This statement is

supported by many researches and articles which put more emphasize on the effect of ionic liquids constituents on their nature (Alexandra et al., 2015; Gouveia et al., 2014; Ventura et al., 2012). And as presented in Table 4.2, the amino acid based anion in the structure of ILs was highly enable of improving the toxicity of the ILs, although it also depends on the type of anion as another factor.

Consistent with the results obtained from toxicity assessment, it can be concluded that the *Staphylococcus aureus* which is a gram-positive bacteria has the highest sensibility to ionic liquids of every kind in comparison with other two bacteria. According to Passino and Smith (1987), if we classify the tested ionic liquids based on their toxicity range (203-940 mg/L), all of them would fall into the category of "practically harmless" category for the mentioned bacteria.

On the other hand, as the nature of the gram-negative bacteria supposes, *Escherichia coli* was stronger towards the tested AAILs in comparison with *Staphylococcus aureus*. This can be clearly seen in the respective EC_{50} value of every tested AAILs.

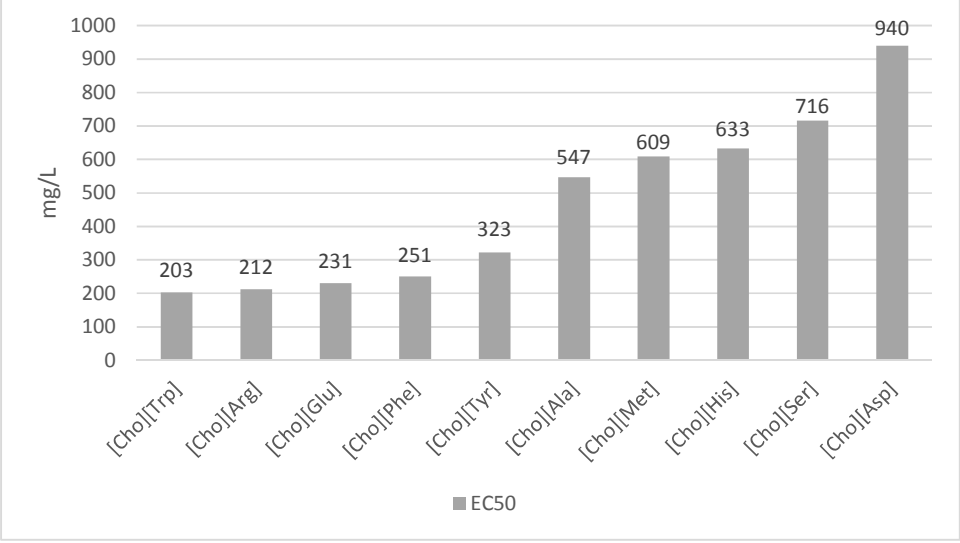


FIGURE 4.3 EC_{50} values of AAILs towards Staphylococcus aureus

As the results of biocompatibility test with *Staphylococcus aureus* suggest (table-4.3) , [Cho][Trp] shows a higher toxicity with an EC_{50} value of 203 mg/L compared to other AAILs, which has the biggest anion molecule among all, then followed by [Cho][Arg] and [Cho][Glu] with a value of 212 and 231 mg/L respectively. The least toxic ionic liquid is found to be [Cho][Asp] with EC_{50} value of 940 mg/L before [Cho][Ser] (716 mg/L).

It is necessary to mention that none of the tested AAILs are absolutely harmless since none of them have an EC50 value of over 1000 mg/L, but they can be considered as "practically harmless" (>100 mg/L) to *Staphylococcus aureus* which is the most sensible bacterium in this study.

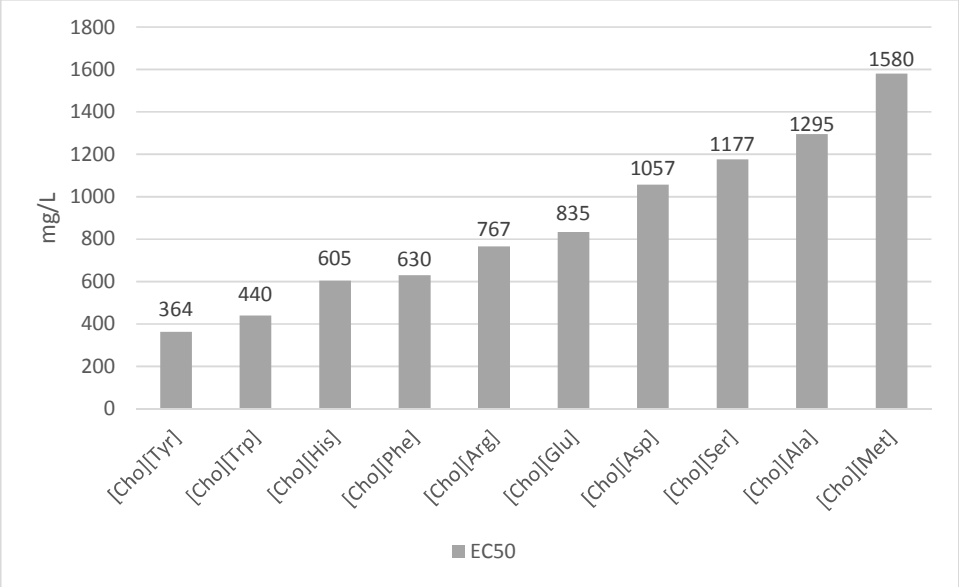


FIGURE 4.4 EC_{50} values of AAILs towards Listeria monocytogenes

The results of AAILs' EC_{50} value in contact with *Listeria monocytogenes* are considerably higher in many cases compared to *Staphylococcus aureus,* even though both

of them are gram-positive bacteria. As well as the previous test [Cho][Tyr] and [Cho][Trp] are demonstrating the most toxic behavior compared to others based on their EC_{50} value which are 364 mg/L and 440 mg/L respectively, and to be categorized as practically harmless. The other three remaining AAILs, [Cho][Met] (1580 mg/L), [Cho][Ala] (1295 mg/L), [Cho][Ser] (1177 mg/L) and [Cho][Asp] (1057 mg/L) have an EC_{50} value of higher than 1000 mg/L which are branded as harmless to *Listeria monocytogenes*.

By comparing the EC_{50} values and the size of the anion molecules in the structure of AAILs, it can be seen that the smaller sizes of the anion, reduced the toxicity of the AAILs to an appreciable extent. This statement is also proved by Hou et.al. (2013). As the anion molecular size increases either by chain length or presence of functional groups, the EC_{50} value of that AAILs decreases. Therefore, the smaller anion molecules in AAILs, make them of more biocompatible types.

The toxicity mechanisms of ILs on bacteria are not fully understood, but it has been suggested that the mode of action happens through membrane disruption, accumulation and surface activity with subsequent accumulation within cells.

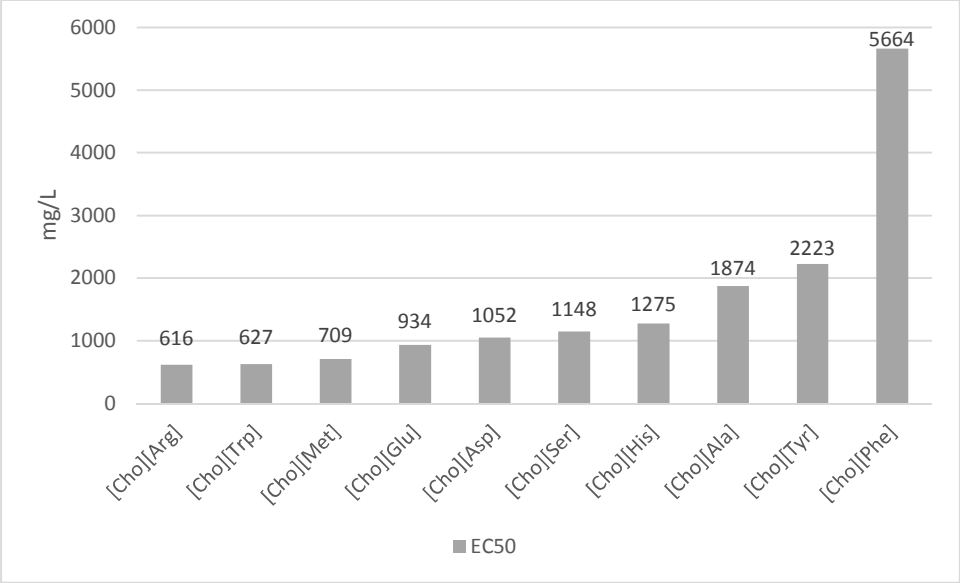


FIGURE 4.5 EC_{50} values of AAILs towards Escherichia coli

The results of toxicity test show that, the effect of AAILs on a gram-negative bacterium is considerably lower than the gram-positive bacteria as presented in table-4.5. By incubating the same AAILs with *Escherichia coli* (gram-negative), majority of the tested AAILs show an EC_{50} value of higher than 1000 mg/L and shall be reported as harmless. Surprisingly, unlike the gram-positive bacteria, [Cho][Phe](5664 mg/L) and [Cho][Tyr] (2223 mg/L) show a great biocompatibility with gram-negative bacteria. The effect of [Cho][Ala] (1874 mg/L) and [Cho][Asp] (1052 mg/L) on this type of bacteria are very similar to their effect on *Listeria monocytogenes.*

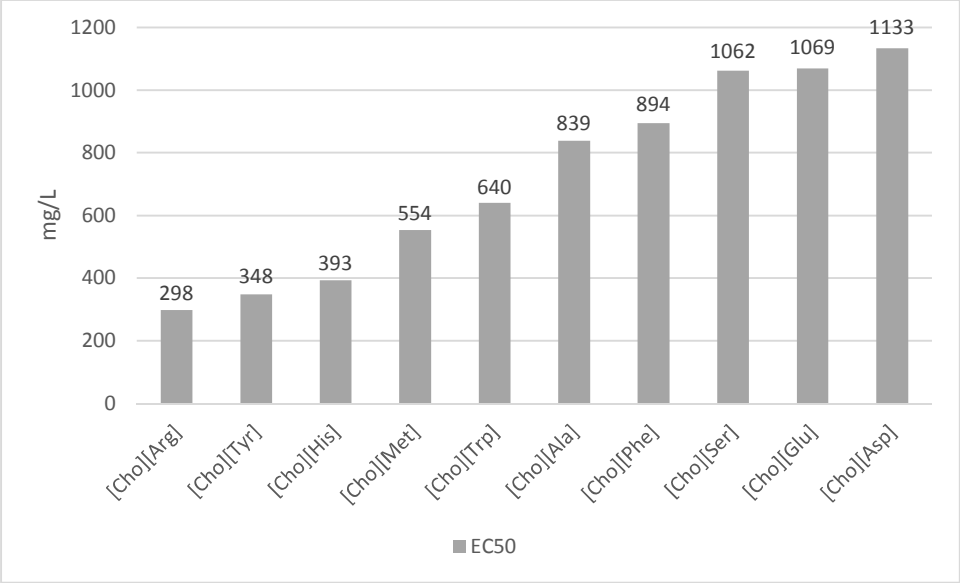


FIGURE 4.6 EC_{50} values of AAILs towards *Aeromonas Hydrophila*

Similar results were obtained by conducting toxicity test of AAILs on *Aeromonas Hydrophila*, a gram-negative bacterium. The same relationship between the toxicity of AAILs and the size of anion is observed. Comparing to *Escherichia Coli*, a similar type of bacteria, EC_{50} values are lower generally for all the AAILs compared to this case. This signifies that that *Aeromonas Hydrophila* is more sensitive in comparison and therefore AAILs Tend to have higher toxicity degree. However all the tested AAILs are at least practically harmless and some of them ([Cho][Ser], [Cho][Glu], [Cho][Asp]) are found to be absolutely harmless.

The EC_{50} values are relatively higher, meaning they are less toxic towards *Escherichia coli* and *Aeromonas Hydrophila*. This could be due to the presence of an outer peptidoglycan layer adjutant to the cytoplasmic membrane of the cell. This scenario decreases permeability of the ILs into the cells, making it able to withstand the presence of ILs into its surroundings.

4.3 Biodegradability Test

Following the procedure described by OECD 301, the dilution water was prepared, minerals added and mixed with AAIL inside the BOD bottles. Two replicates were prepared for each substance of test, and since it is expected to have a high degree of biodegradation, the samples were introduced with a dilution factor of 10 and oxygen demand limit of 2000 mg/L, manipulated in the settings of OxiTop apparatus.

An important part of biodegradability test is the location of seeding water source since different sources such as industrial sewage water, pond water or river have different types of microorganisms which may differ the test results for different applications. In this test seeding water samples were taken from two sources of a lake(latitude 4.381675˚N and longitude 100.979036˚E) and river mixed with industrial sewage water (latitude 4.387507˚N and longitude 100.973325˚E)

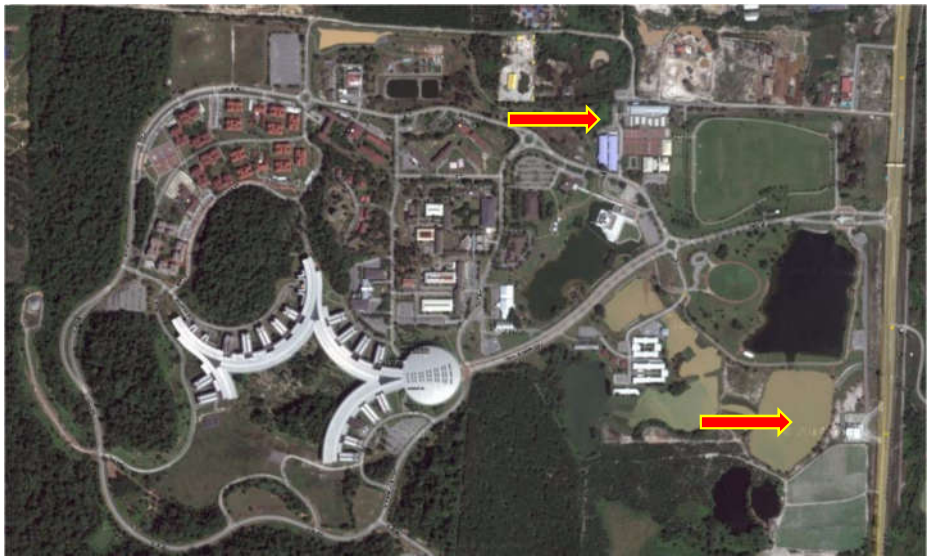


FIGURE 4.7 Geographical location of the seeding water samples

In order to prevent stagnancy of biological activity or death of bacteria inside the test bottles, the seeding water with the highest number of bacteria was chosen. In order to find out the amount of bacteria in each water sample, 10µl of each of the samples were placed on an agar solution separately and incubated for 24h. By looking at the petri

dishes, the water sample with the higher number of microorganisms could be easily identified.

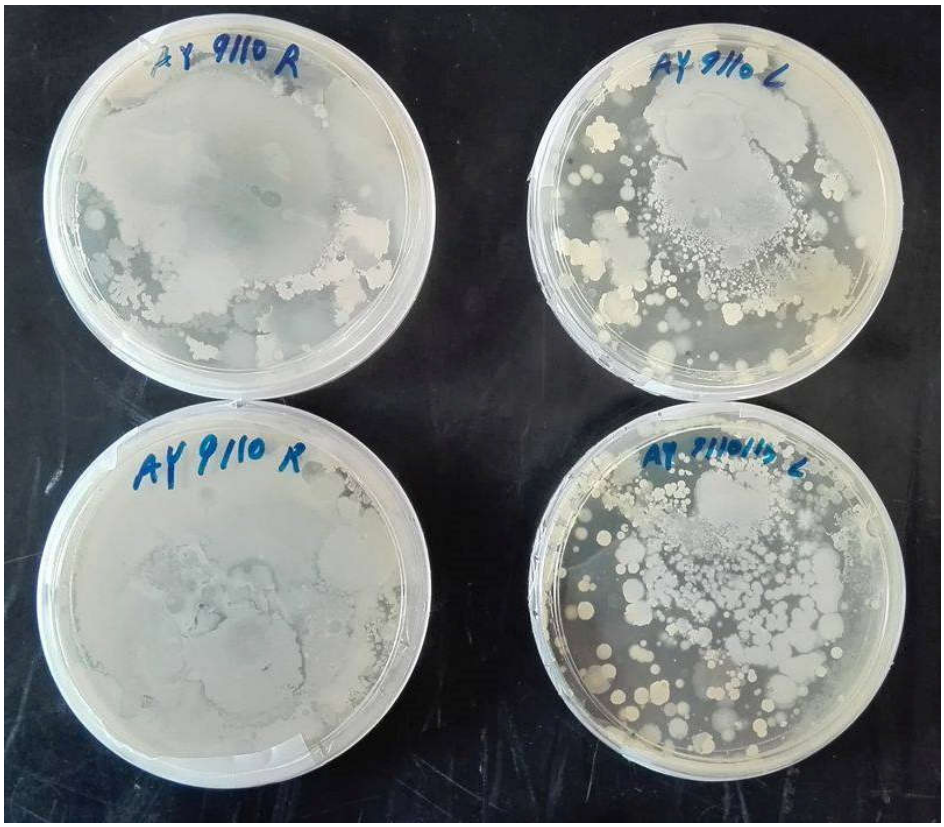


FIGURE 4.8 Growth of seeding water microorganisms, Sewage (left) and Lake (right)

Figure-7 clearly shows that the microorganisms in the sewage water are presented in greater number and intensity, therefore this source of water was chosen for the biodegradability test.

Following the procedure of closed bottle test for chemical substances, the biodegradability of the AAILs found as presented in the table below:

Table 4.3 Biodegradability of the synthesized AAILs.

Entry	Ionic Liquid	Biodegradability (%), 28 days
1	[Cho][Ala]	64.1 ± 2.2
2	[Cho][Met]	65.1 ± 3.9
3	[Cho][Ser]	71.1 ± 3.2
4	[Cho][His]	72.5 ± 3.0
5	[Cho][Tyr]	72.8 ± 2.6
6	[Cho][Phe]	73.8 ± 1.8
7	[Cho][Asp]	77.0 ± 5.5
8	[Cho][Arg]	77.3 ± 4.5
9	[Cho][Glu]	83.5 ± 2.0
10	[Cho][Trp]	85.8 ± 9.4

Average data are shown with ± SD (n=2)

As it was expected from biomaterials derived ionic liquids to show a high biodegradability, the results of the biodegradability experiment indicate this as well. Based on the level of mineralization of AAILs through microbial breakdown which is above 60% in all the cases, they can be referred as “readily biodegradable”, according to Organization for Economic Cooperation and Development, OECD, (2001). Readily biodegradable substances go through biodegradation in wastewater treatment plants or in natural water reservoirs within short terms and they are not persistent; this is a certification which claims they are “green” materials.

Different biodegradability values indicates that the type of anion incorporated in the structure of AAILs has a vital effect in determining the quality of biodegradation. The other fact that can be extracted from the data is that, biodegradability of the AAILs improved as the size of the anion molecule increased. For bigger anion molecules such

as L-Glutamine, L-Tryptophan and L-Phenylalanine which contain a benzene ring as a functional group in their molecule, the biodegradability was the highest. This is because the amount of oxygen required by the microbial consortia to degrade the larger compounds are higher.

Longer chain length of the anion structure also helped with a higher biodegradability range. Comparing [Cho][Ala] and [Cho][Asp], as the later one has a longer chain, it appears to have higher biodegradability (77.0%) compared to L-Alanine (64.1%) which has the smallest molecule size among all. Presence of thioether functional group in [Cho][Met] might be reason of lower biodegradability of this molecule compared to others.

CHAPTER 5
CONCLUSION AND RECOMMENDATION

Increasing attention has been developed towards ILs since 1990s due to their impressive physicochemical properties and as green substitutes of conventional solvents in industries. But only of recent that their toxicity have been challenged by many researches. Later, it became proven that ILs derived from biomaterial show a better biodegradability. In this respect, it is appreciable to find a relationship between the toxicity of ILs and their structure, as there is a possibility to synthesize them for task oriented pharmaceutical and industrial applications.

In this project, the effect of anion in the choline based ILs are being studied to analyze how different anions with the same cation ([Cho]) in ILs change their biocompatibility and biodegradability characteristics through eco-toxicity tests. Synthesizing of ionic liquids achieved with different yields. While better dissolution of amino acid provides a higher reaction rate and as a result, higher yield.

Eco-toxicity results of AAILs revealed that, different anions are imposing different effects on the microorganisms. The molecular structure of anion can be taken into analysis to justify their effect. It was found that bigger anion molecules behave more poisonous and smaller anion sizes improve the biocompatibility. It is necessary to mention that the type of microorganism is also an important factor in analyzing and concluding the toxicity of AAILs since each of the gram-negative and gram-positive bacteria showed different reactions to an individual IL. As it was expected from choline based amino acid ionic liquids to improve the toxicity, the obtained results met this expectation confirming that these kinds of ILs are at least practically harmless to various bacteria. The effect of anion molecule size in biodegradability concluded to have direct

effect, while larger the anion molecule, greater the biodegradability. Therefore, the anion molecule size becomes counter wise in favor of improving biocompatibility and biodegradability. Compromises should be made between biodegradability and biocompatibility in order to select an anion.

Further analysis and researches could be carried out to explore more biomedical applications of AAILs as they were attested to be safe towards a series of bacteria which resembles environmental consortium. If AAILs verified to be harmless on cells this could be a step towards another field of pharmaceutical applications such as drug delivery, lubricants, biocides and etc. A stronger understanding of the properties of ionic liquids especially their toxicity, possibly will help researchers in designing more useful and applicable ionic liquids. Perhaps this approach shall be a platform towards an improved designing of task specified ionic liquids.

REFERENCES

Balk, A., Holzgrabe, U., & Meinel, L. (2015). 'Pro et contra'ionic liquid drugs–Challenges and opportunities for pharmaceutical translation. *European Journal of Pharmaceutics and Biopharmaceutics*, *94*, 291-304.

Costa, A., Forte, A., Zalewska, K., Tiago, G., Petrovski, Z., & Branco, L. C. (2015). Novel biocompatible ionic liquids based on gluconate anion. *Green Chemistry Letters and Reviews*, *8*(1), 8-12.

Davis, S., Hada, S., Herring, R., & Eden, M. (2014). Characterization Based Reverse Design of Ionic Liquids. *Computer Aided Chemical Engineering*, 285-290.

Docherty, K. M., Dixon, J. K., & Kulpa Jr, C. F. (2007). Biodegradability of imidazolium and pyridinium ionic liquids by an activated sludge microbial community. *Biodegradation*, *18*(4), 481-493.

Gavhane, K. A. (2012). Introduction to Process Calculations Stoichiometry. Nirali PRAKASHAN.

Gouveia, W., Jorge, T. F., Martins, S., Meireles, M., Carolino, M., Cruz, C., & Araújo, M. E. M. (2014). Toxicity of ionic liquids prepared from biomaterials. *Chemosphere*, *104*, 51-56.

Hu, S., Jiang, T., Zhang, Z., Zhu, A., Han, B., Song, J., & Li, W. (2007). Functional ionic liquid from biorenewable materials: synthesis and application as a catalyst in direct aldol reactions. *Tetrahedron letters*, *48*(32), 5613-5617.

Kagimoto, J., Taguchi, S., Fukumoto, K., & Ohno, H. (2010). Hydrophobic and low-density amino acid ionic liquids. *Journal of Molecular Liquids*, *153*(2), 133-138.

Egorova, K. S., Seitkalieva, M. M., Posvyatenko, A. V., & Ananikov, V. (2014). Unexpected increase of toxicity of amino acid-containing ionic liquids. *Toxicol. Res.*

Kumar, V., & Malhotra, S. V. (2010). Ionic liquids as pharmaceutical salts: a historical perspective. In *ACS symposium series* (Vol. 1038, pp. 1-12). Oxford University Press.

Lopes, J. M., Paninho, A. B., Môlho, M. F., Nunes, A. V., Rocha, A., Lourenço, N. M., & Najdanovic-Visak, V. (2013). Biocompatible choline based ionic salts: Solubility in short-chain alcohols. *The Journal of Chemical Thermodynamics*, *67*, 99-105.

Markiewicz, M., Piszora, M., Caicedo, N., Jungnickel, C., & Stolte, S. (2013). Toxicity of ionic liquid cations and anions towards activated sewage sludge organisms from different sources–consequences for biodegradation testing and wastewater treatment plant operation. *Water research*, *47*(9), 2921-2928.

Neumegen, R. A., Fernandez-Alba, A. R., & Chisti, Y. (2005). Toxicities of triclosan, phenol, and copper sulfate in activated sludge. *Environmental toxicology*, *20*(2), 160-164.

Class, A. O. T. A. T. (2001). OECD GUIDELINE FOR TESTING OF CHEMICALS.

Ohno, H., & Fukumoto, K. (2007). Amino acid ionic liquids. *Accounts of chemical research*, *40*(11), 1122-1129.

Passino, D. R., & Smith, S. B. (1987). Acute bioassays and hazard evaluation of representative contaminants detected in Great Lakes fish. *Environmental Toxicology and Chemistry*, *6*(11), 901-907.

Pisarova, L., Steudte, S., Dörr, N., Pittenauer, E., Allmaier, G., Stepnowski, P., & Stolte, S. (2012). Ionic liquid long-term stability assessment and its contribution to toxicity and biodegradation study of untreated and altered ionic liquids. *Proceedings of the Institution of Mechanical Engineers, Part J: Journal of Engineering Tribology*, *226*(11), 903-922.

Posten, C. H., & Cooney, C. L. (1993). Growth of microorganisms. *Biotechnology Set, Second Edition*, 111-162.

Romero, A., Santos, A., Tojo, J., & Rodriguez, A. (2008). Toxicity and biodegradability of imidazolium ionic liquids. *Journal of Hazardous Materials*, *151*(1), 268-273.

Tang, F., Zhang, Q., Ren, D., Nie, Z., Liu, Q., & Yao, S. (2010). Functional amino acid ionic liquids as solvent and selector in chiral extraction. *Journal of Chromatography A*, *1217*(28), 4669-4674.

Trivedi, T. J., Rao, K. S., Singh, T., Mandal, S. K., Sutradhar, N., Panda, A. B., & Kumar, A. (2011). Task-Specific, Biodegradable Amino Acid Ionic Liquid Surfactants. *ChemSusChem*, *4*(5), 604-608.

Ventura, S. P., Marques, C. S., Rosatella, A. A., Afonso, C. A., Gonçalves, F., & Coutinho, J. A. (2012). Toxicity assessment of various ionic liquid families towards Vibrio fischeri marine bacteria. *Ecotoxicology and environmental safety*, *76*, 162-168.

APPENDICES

Appendices A H-NMR spectrum of the synthesized AAILs

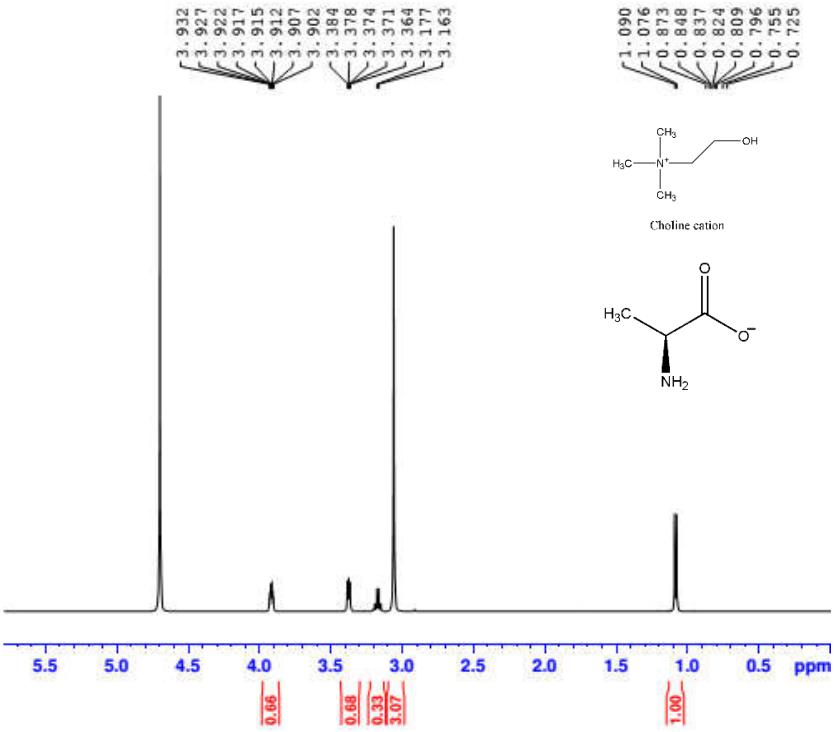


FIGURE A1 H-NMR spectrum of [Cho][Ala]

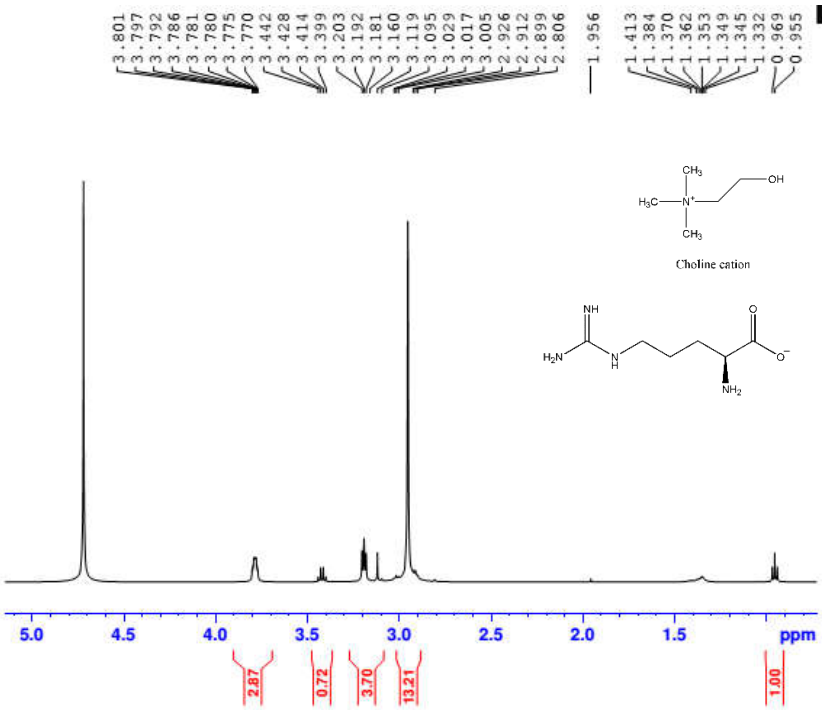


FIGURE A2 H-NMR spectrum of [Cho][Arg]

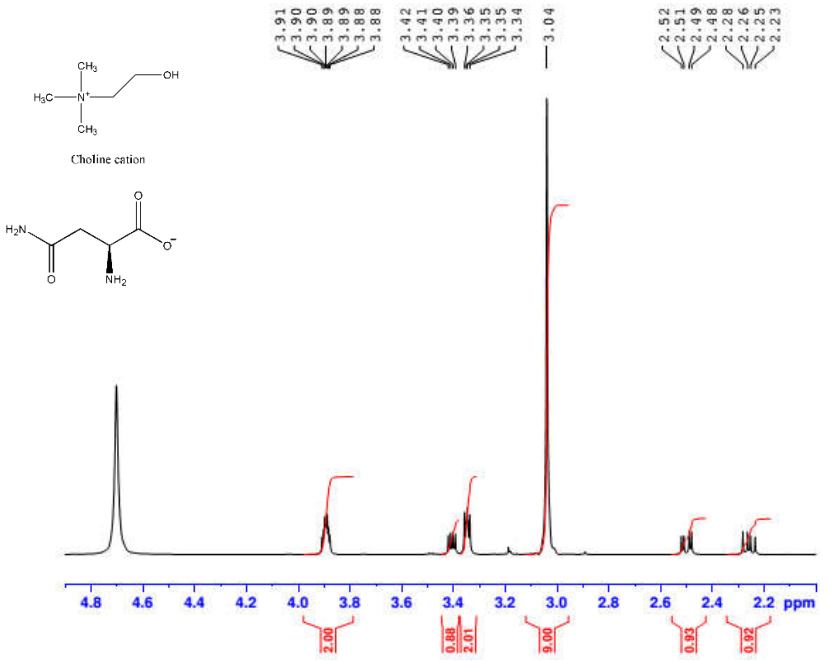


FIGURE A3 H-NMR spectrum of [Cho][Asp]

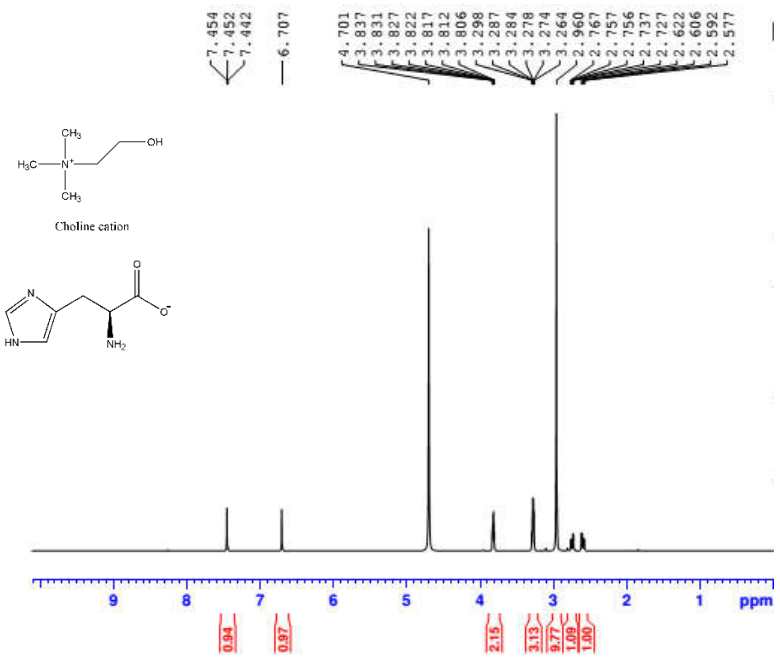


FIGURE A4 H-NMR spectrum of [Cho][His]

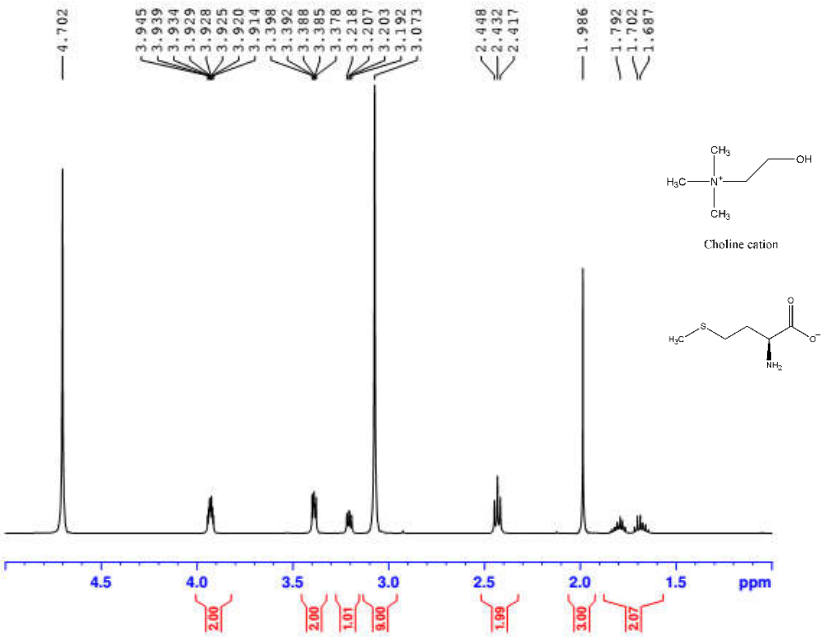


FIGURE A5 H-NMR spectrum of [Cho][Met]

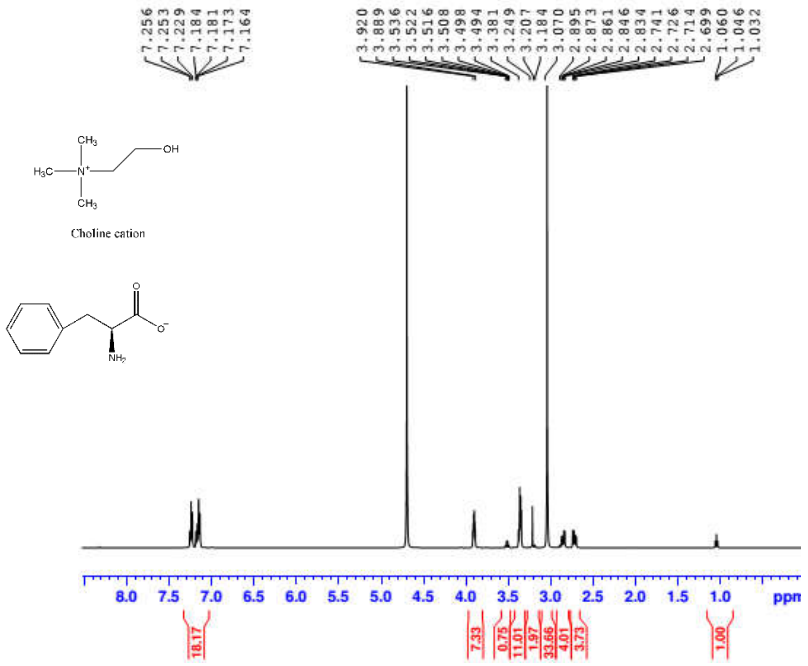


FIGURE A6 H-NMR spectrum of [Cho][Phe]

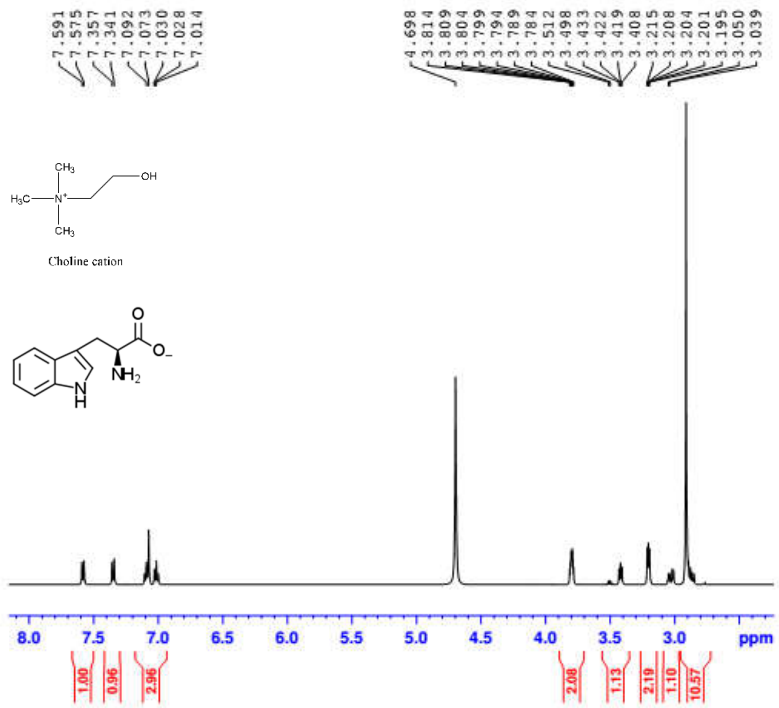


FIGURE A7 H-NMR spectrum of [Cho][Trp]

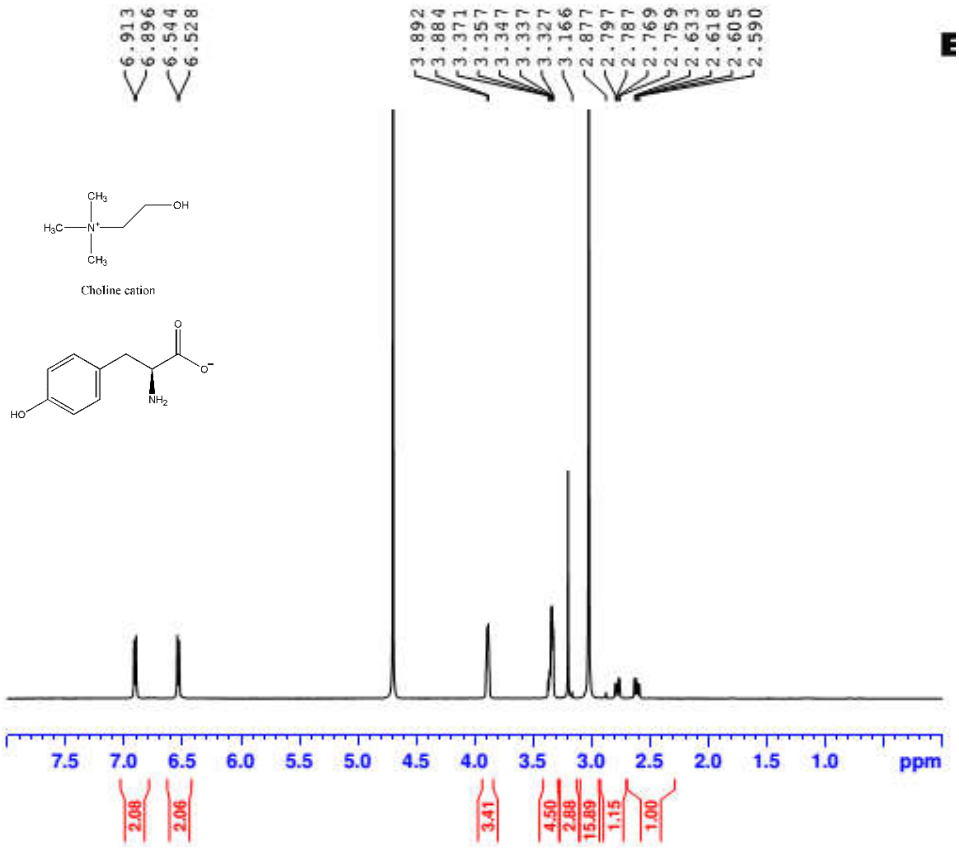


FIGURE A8 H-NMR spectrum of [Cho][Tyr]

Appendices B: Thermal Gravimetric Analysis (TGA) results

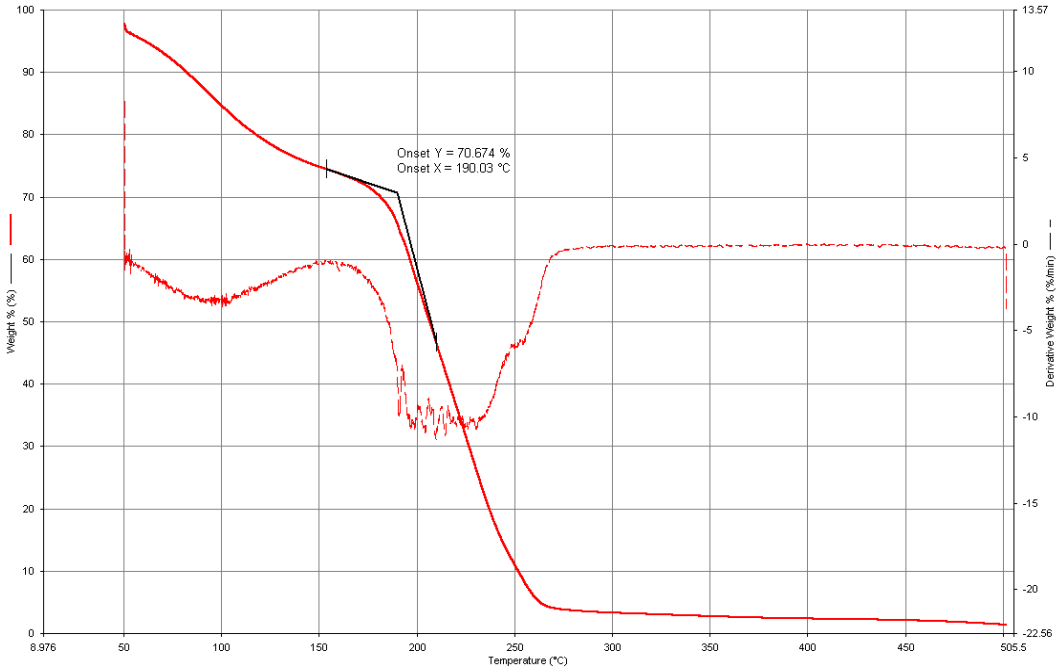


FIGURE B1 [Cho][Ala] decomposition temperature

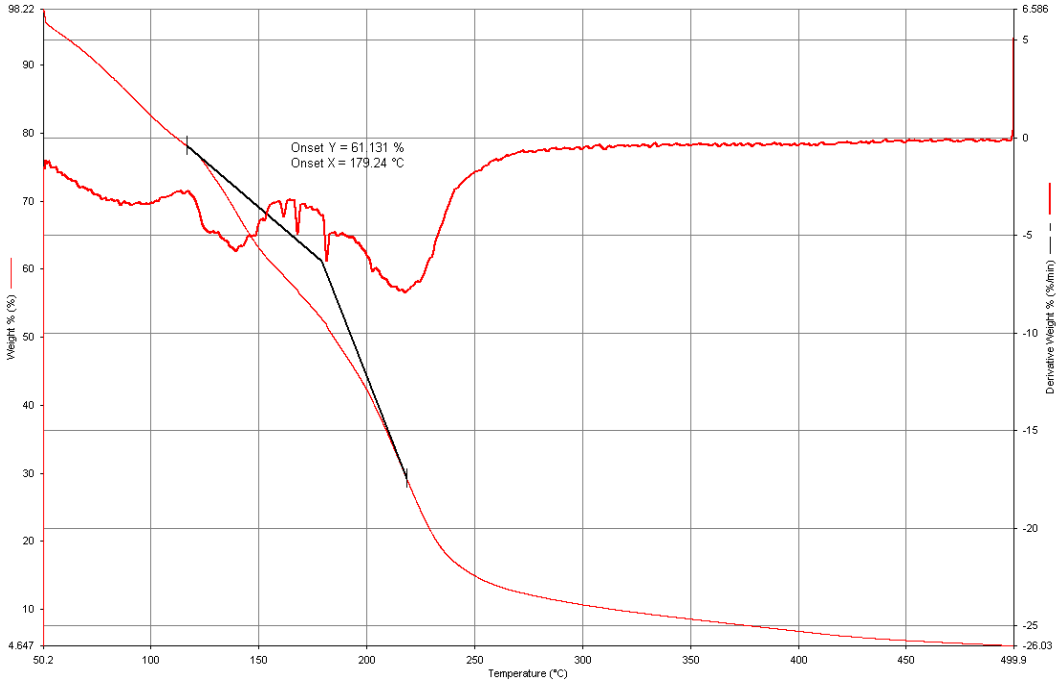


FIGURE B2 [Cho][Arg] decomposition temperature

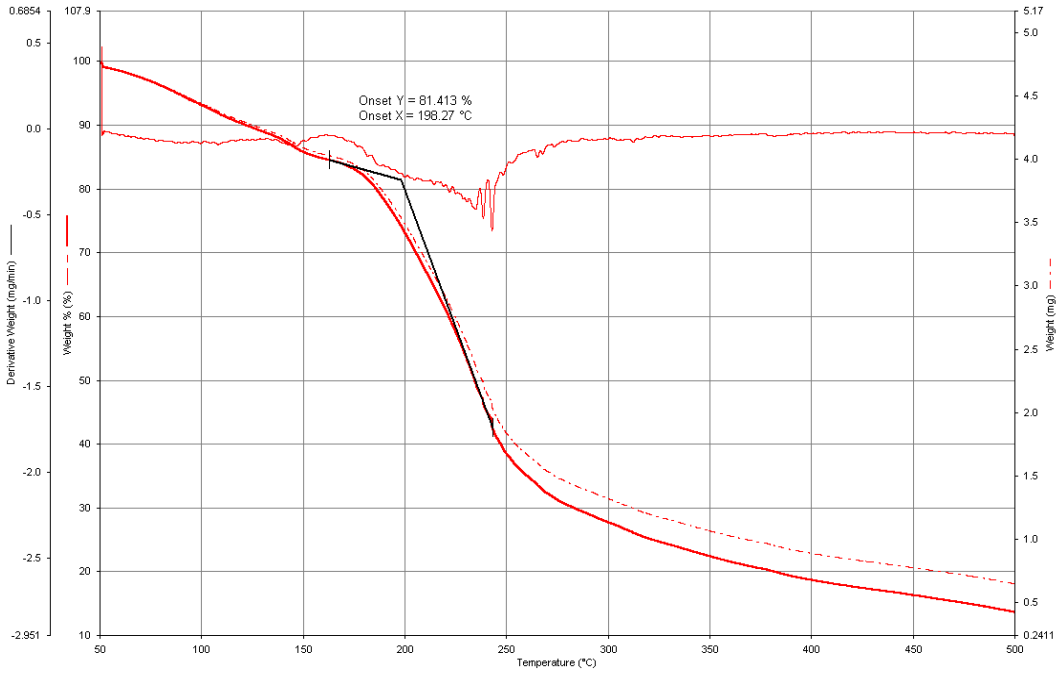


FIGURE B3 [Cho][Asp] decomposition temperature

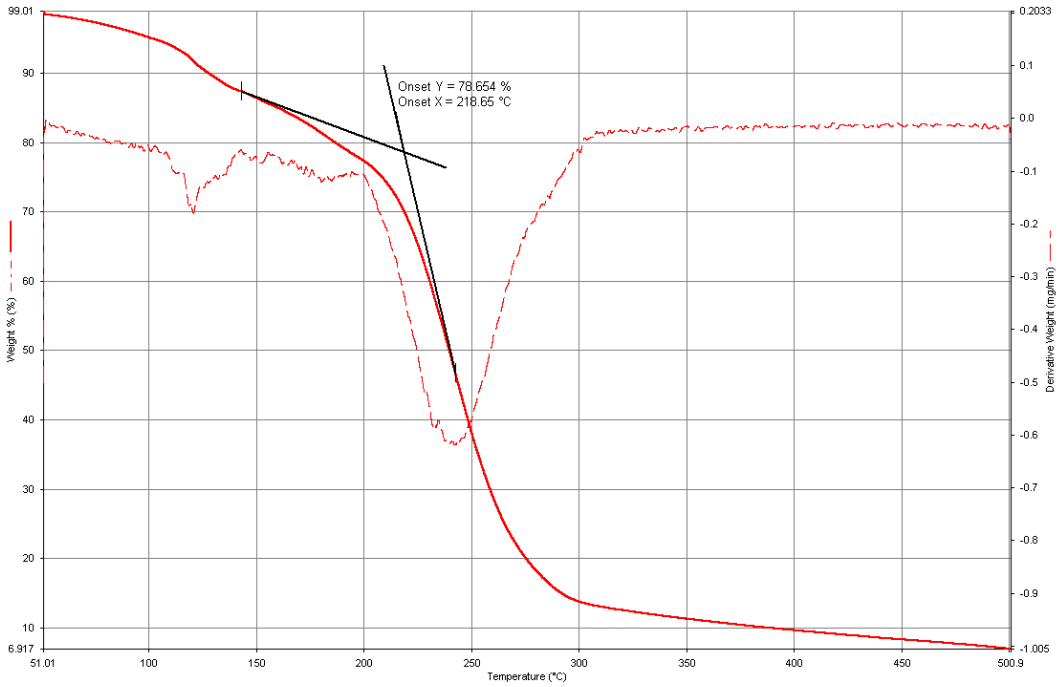


FIGURE B4 [Cho][Glu] decomposition temperature

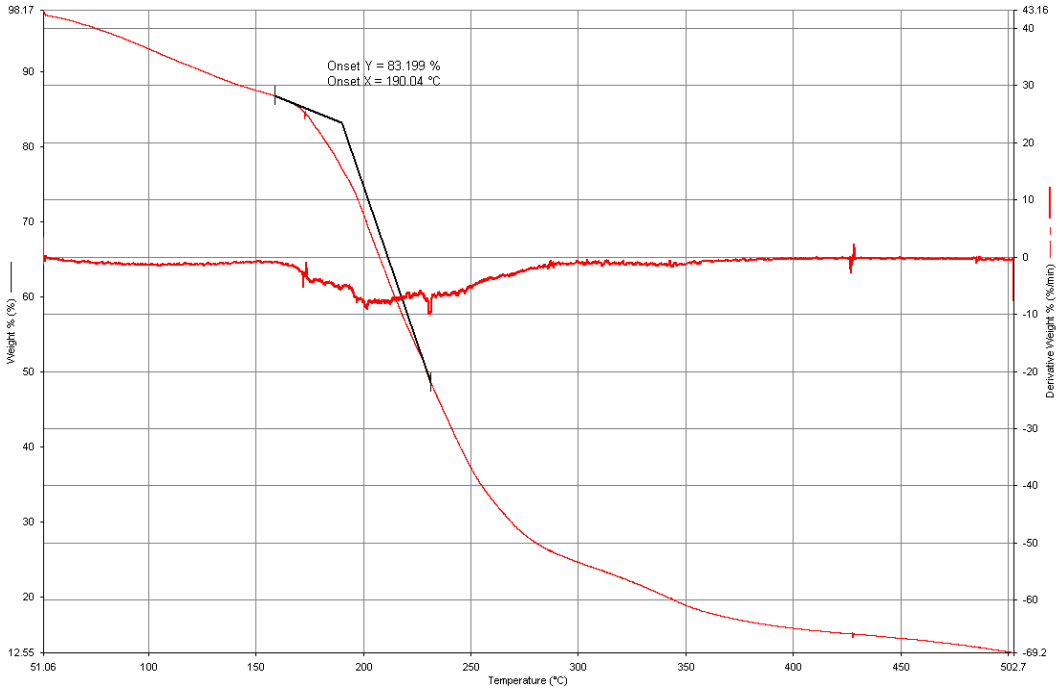


FIGURE B5 [Cho][His] decomposition temperature

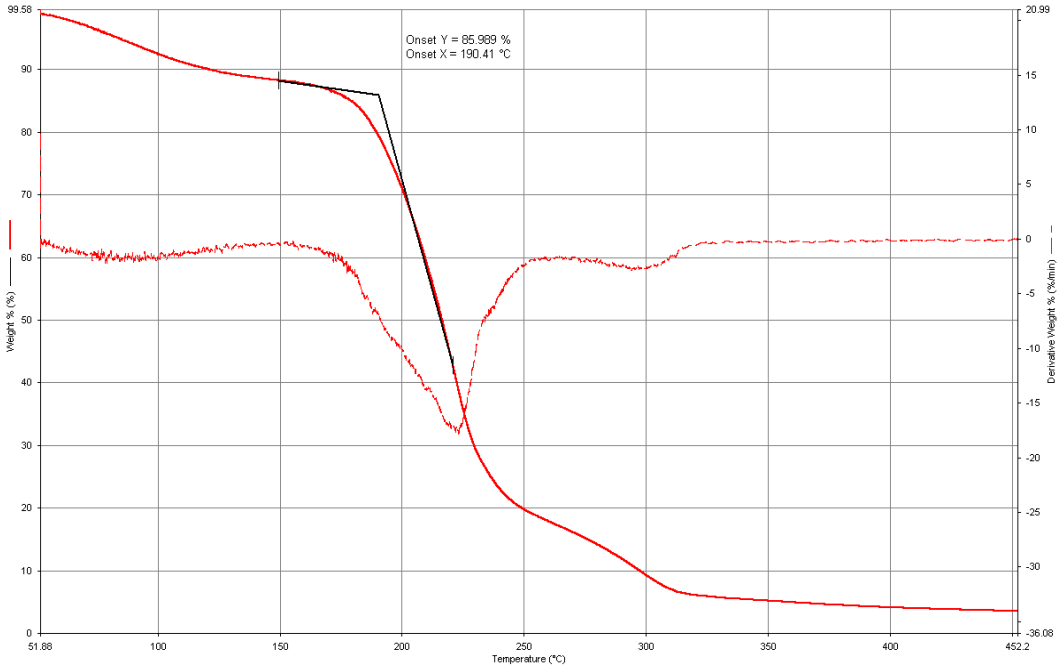


FIGURE B6 [Cho][Met] decomposition temperature

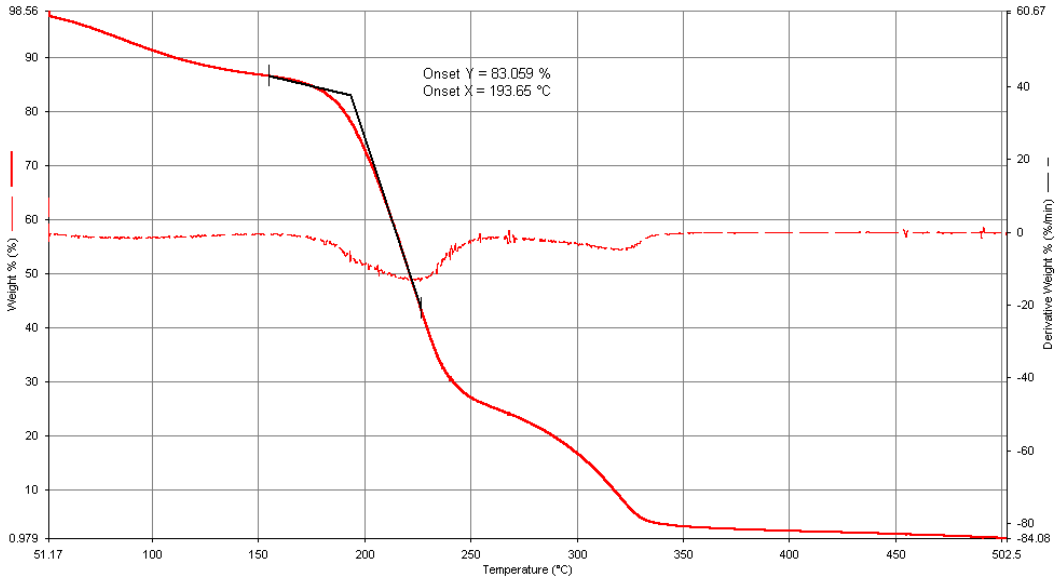


FIGURE B7 [Cho][Phe] decomposition temperature

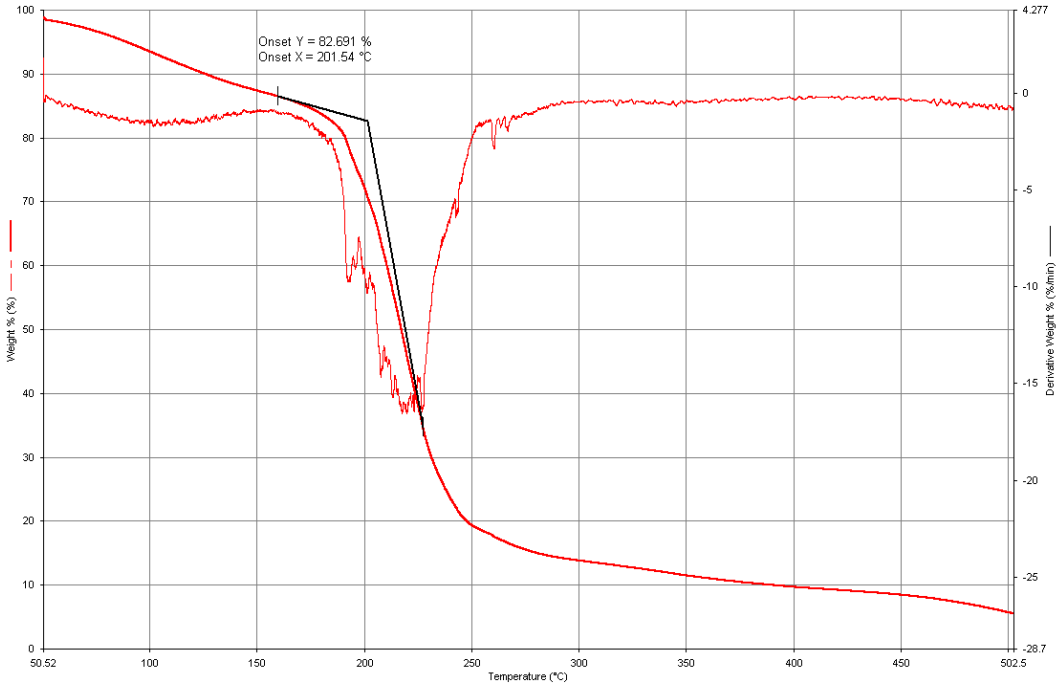


FIGURE B8 [Cho][Ser] decomposition temperature

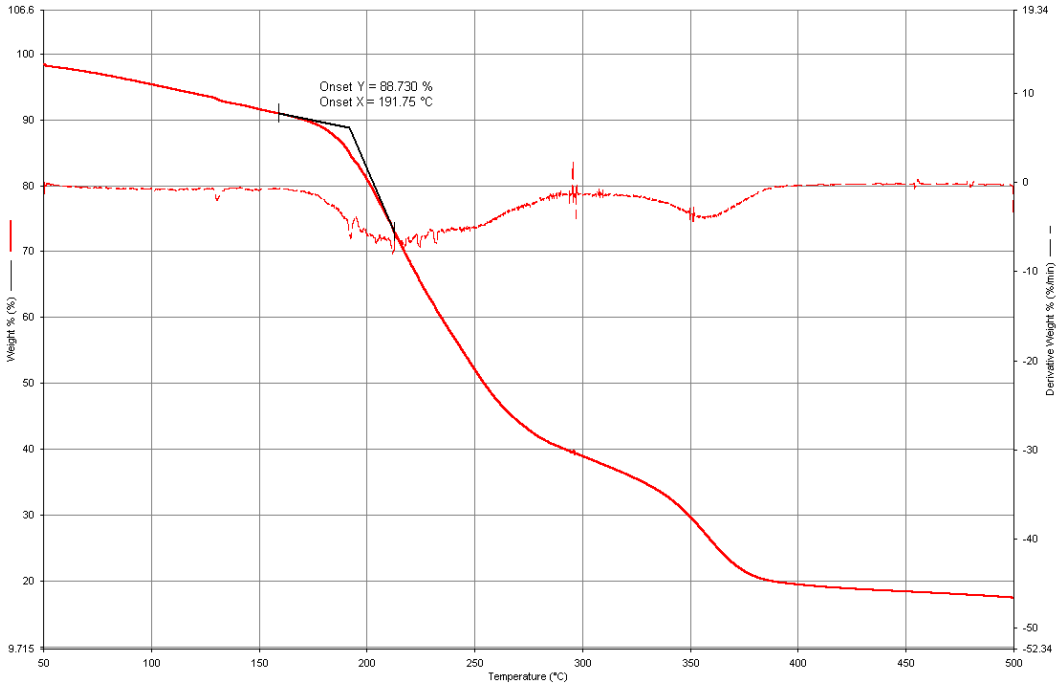


FIGURE B9 [Cho][Trp] decomposition temperature

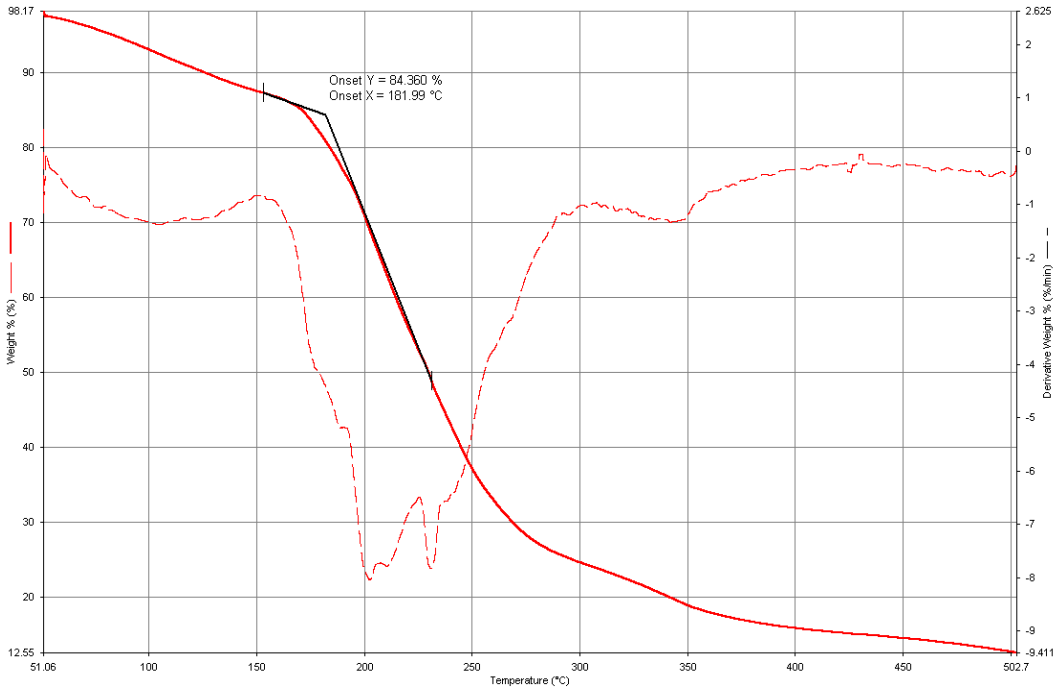


FIGURE B10 [Cho][Tyr] decomposition temperature

Appendices C Normalize of transform of dose- response curve

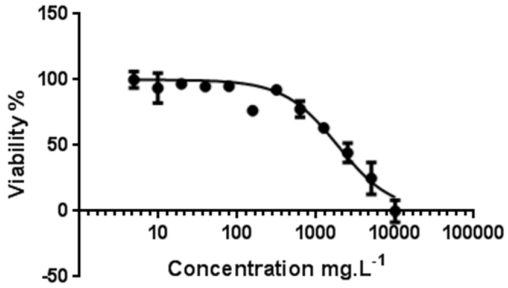


FIGURE C1 Normalize of Transform of dose vs. response, [Cho][Ala] towards E.C.

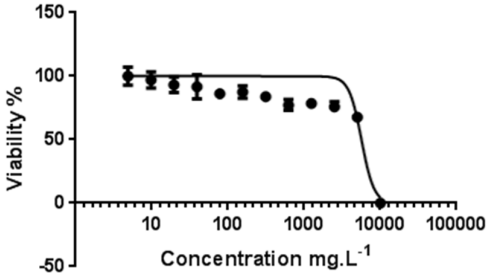


FIGURE C2 Normalize of Transform of dose vs. response, [Cho][Phe] towards E.C.

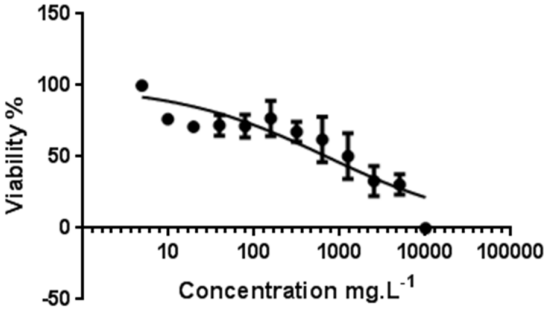


FIGURE C3 Normalize of Transform of dose vs. response, [Cho][Met] towards E.C.

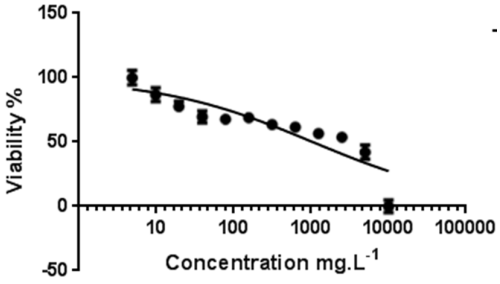


FIGURE C4 Normalize of Transform of dose vs. response, [Cho][Asp] towards E.C.

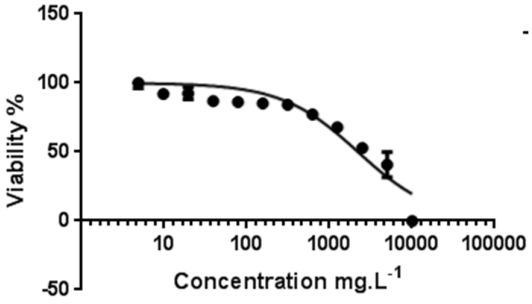


FIGURE C5 Normalize of Transform of dose vs. response, [Cho][Tyr] towards E.C.

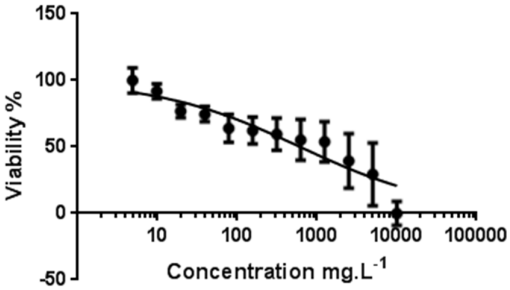


FIGURE C6 Normalize of Transform of dose vs. response, [Cho][Arg] towards E.C.

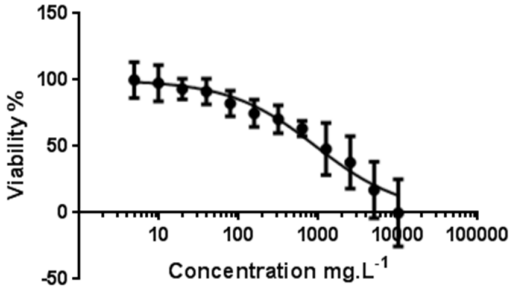


FIGURE C7 Normalize of Transform of dose vs. response, [Cho][Glu] towards E.C.

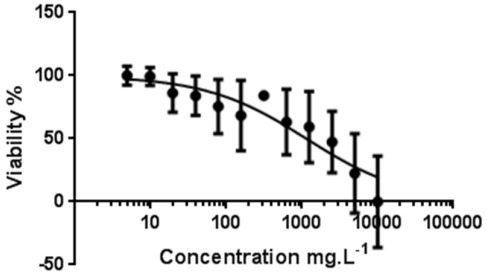


FIGURE C8 Normalize of Transform of dose vs. response, [Cho][Ser] towards E.C.

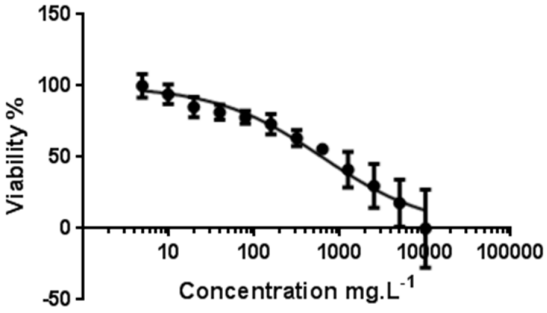


FIGURE C9 Normalize of Transform of dose vs. response, [Cho][Trp] towards E.C.

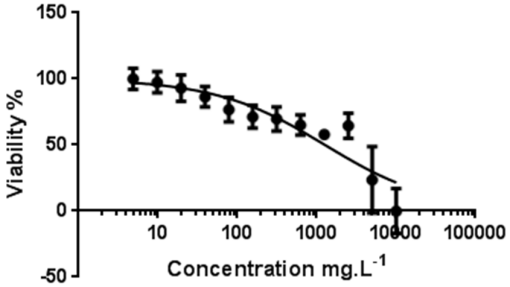


FIGURE C10 Normalize of Transform of dose vs. response, [Cho][His] towards E.C.

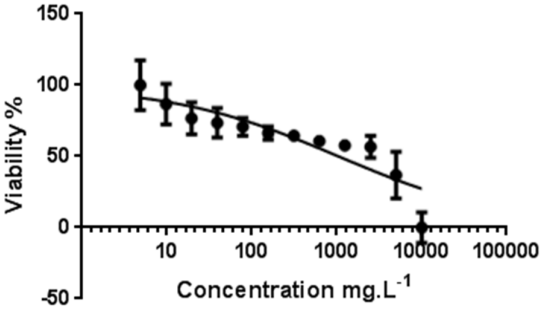


FIGURE C11 Normalize of Transform of dose vs. response, [Cho][Ala] towards L.M.

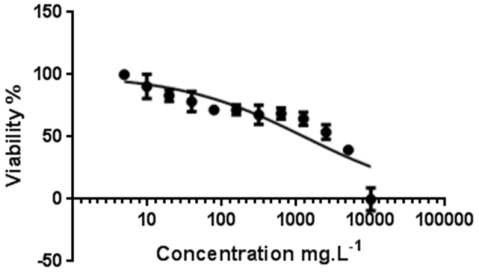


FIGURE C12 Normalize of Transform of dose vs. response, [Cho][Phe] towards L.M.

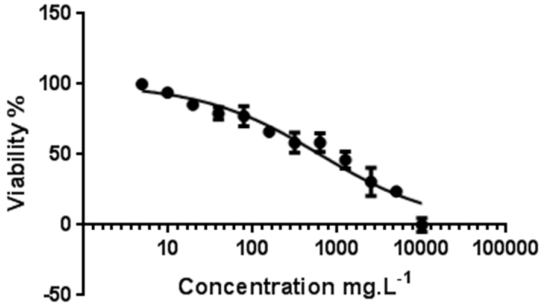


FIGURE C13 Normalize of Transform of dose vs. response, [Cho][Met] towards L.M.

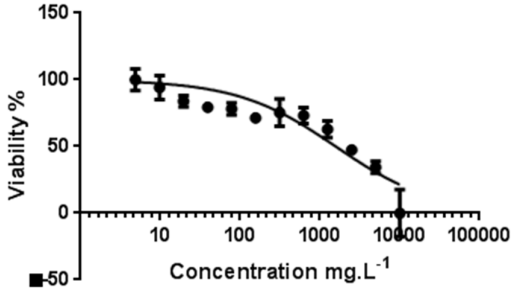


FIGURE C14 Normalize of Transform of dose vs. response, [Cho][Asp] towards L.M.

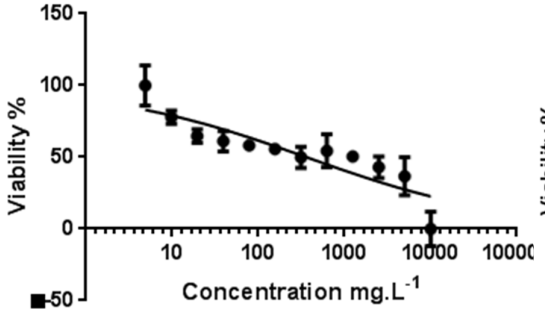


FIGURE C15 Normalize of Transform of dose vs. response, [Cho][Tyr] towards L.M.

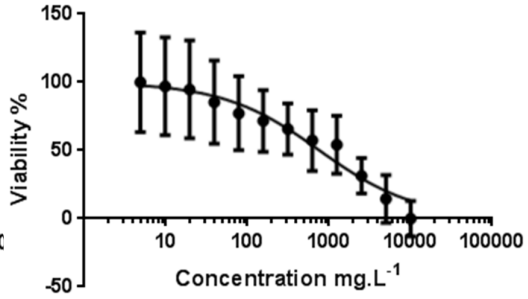


FIGURE C16 Normalize of Transform of dose vs. response, [Cho][Arg] towards L.M.

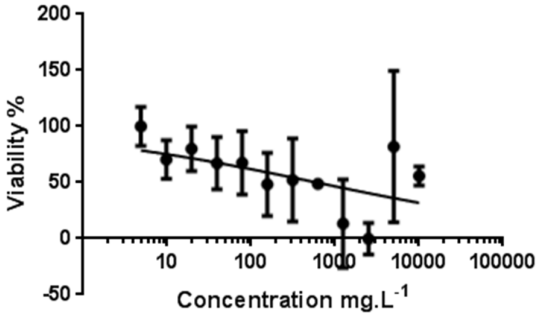


FIGURE C17 Normalize of Transform of dose vs. response, [Cho][His] towards L.M.

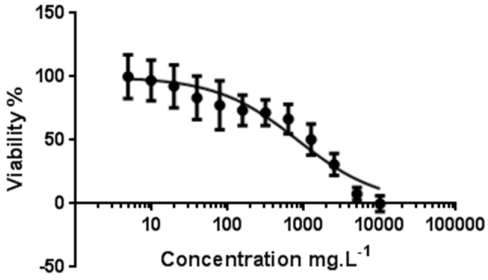


FIGURE C18 Normalize of Transform of dose vs. response, [Cho][Glu] towards L.M.

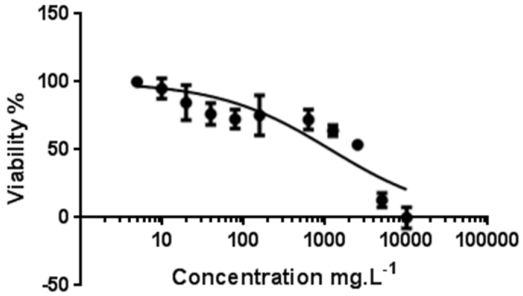


FIGURE C19 Normalize of Transform of dose vs. response, [Cho][Ser] towards L.M.

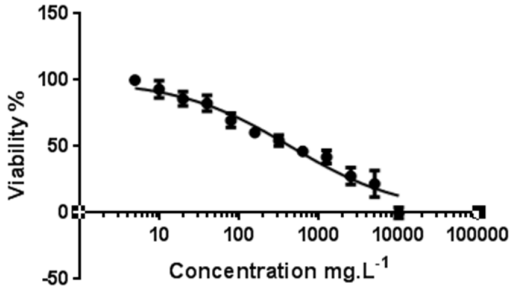


FIGURE C20 Normalize of Transform of dose vs. response, [Cho][Trp] towards L.M.

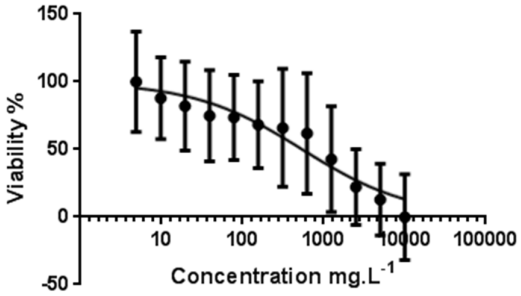


FIGURE C21 Normalize of Transform of dose vs. response, [Cho][Ala] towards S.A.

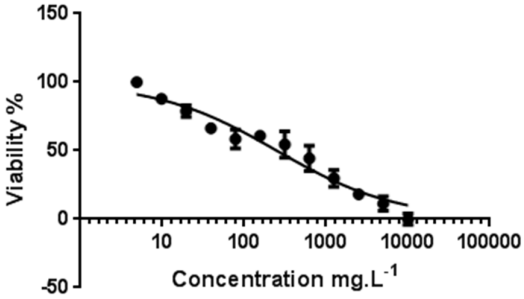


FIGURE C22 Normalize of Transform of dose vs. response, [Cho][Phe] towards S.A.

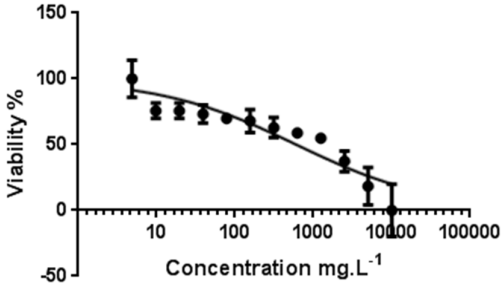


FIGURE C23 Normalize of Transform of dose vs. response, [Cho][Met] towards S.A.

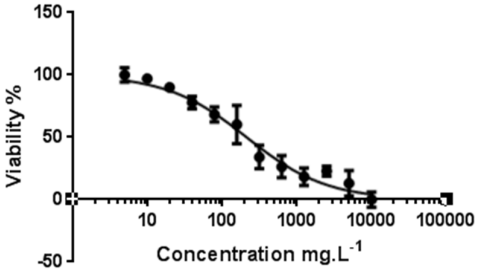


FIGURE C24 Normalize of Transform of dose vs. response, [Cho][Arg] towards S.A.

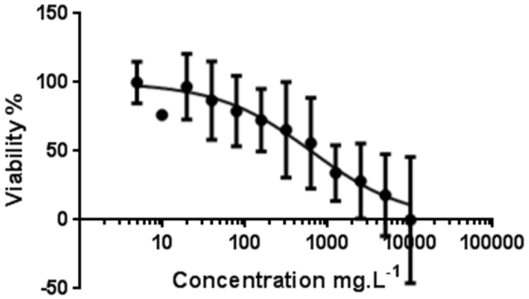


FIGURE C25 Normalize of Transform of dose vs. response, [Cho][His] towards S.A.

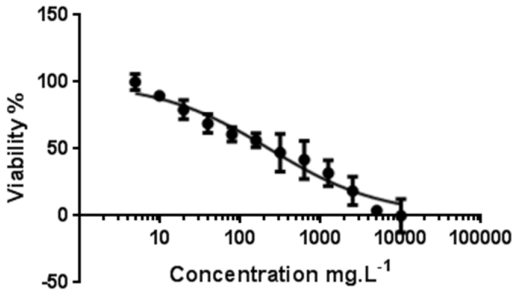


FIGURE C26 Normalize of Transform of dose vs. response, [Cho][Glu] towards S.A.

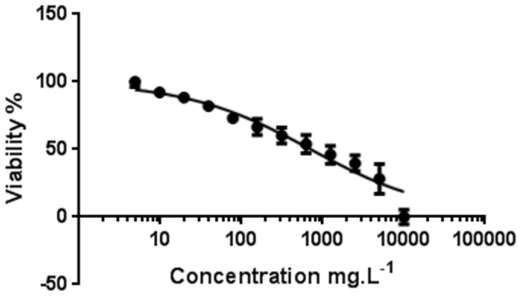


FIGURE C27 Normalize of Transform of dose vs. response, [Cho][Ser] towards S.A.

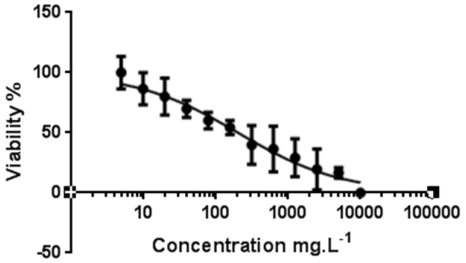


FIGURE C28 Normalize of Transform of dose vs. response, [Cho][Trp] towards S.A.

Appendices D Biodegradability calculations

No.	Ionic liquid	Reading	substance	substance		BOD	Biodegradability	average
		mg/l	mg/43.5ml	mg/1000ml	TheoD		BIOD	
1	[Cho][Asp] 1	325.000	14.830	305.773	1.360	0.994	0.731	0.770
2	[Cho][Asp] 2	367.000	15.230	314.021	1.360	1.101	0.810	
3	[Cho][Tyr] 1	1059.000	36.300	748.454	1.857	1.387	0.747	0.728
4	[Cho][Tyr] 2	621.000	22.060	454.845	1.857	1.319	0.710	
5	[Cho][Ala] 1	381.000	15.970	329.278	1.664	1.093	0.657	0.641
6	[Cho][Ala] 2	331.000	14.430	297.526	1.664	1.041	0.626	
7	[Cho][Met] 1	494.000	18.370	378.763	1.839	1.248	0.679	0.651
8	[Cho][Met] 2	483.000	19.550	403.093	1.839	1.146	0.623	
9	[Cho][Phe] 1	466.000	14.680	302.680	2.027	1.470	0.725	0.738
10	[Cho][Phe] 2	508.000	15.530	320.206	2.027	1.520	0.750	
11	Blank 1	14.100						21.150
12	Blank 2	28.200						
13	[Cho][Arg] 1	566.000	21.980	457.917	1.442	1.159	0.804	0.773
14	[Cho][Arg] 2	467.000	19.380	403.750	1.442	1.069	0.741	
15	[Cho][Trp] 1	933.000	24.180	503.750	1.926	1.782	0.925	0.858
16	[Cho][Trp] 2	1103.000	33.660	701.250	1.926	1.523	0.791	
17	[Cho][His] 1	576.000	23.370	486.875	1.487	1.111	0.747	0.725
18	[Cho][His] 2	562.000	24.160	503.333	1.487	1.046	0.704	
19	[Cho][Ser] 1	763.000	32.610	679.375	1.460	1.071	0.734	0.711
20	[Cho][Ser] 2	438.000	19.210	400.208	1.460	1.006	0.689	
21	[Cho][Glu] 1	523.000	18.660	388.750	1.476	1.255	0.850	0.835
22	[Cho][Glu] 2	425.000	15.440	321.667	1.476	1.212	0.821	
23	Blank 1	42.400						35.300
24	Blank 2	28.200						

Table D.1 Biodegradability of the synthesized AAILs.