An Investigation into the use of Ionic Liquid Solvents as Potential Therapeutic Agents to help Alleviate Disease caused by the Formation of Insoluble Plaque or Depositions

Sundus Alchaar

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An Investigation into the use of Ionic Liquid Solvents as Potential Therapeutic Agents to help Alleviate Disease caused by the Formation of Insoluble Plaque or Depositions

Sundus Alchaar

Submitted in Partial Completion of the Requirements for
Commonwealth Honors in Chemical Sciences

Bridgewater State University

May 9, 2023

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Abstract

Many diseases are caused or worsened by the formation of insoluble aggregates inside the human body, most of which are currently treated by maintenance medication or surgery. Biocompatible Ionic Liquids (ILs) have been explored for use as therapeutic agents, and are also excellent solvents, due to their versatile physical and chemical properties. The aim of this project was to conduct a preliminary exploration of the use of ILs to dissolve different types of depositions, such as kidney stones and crystal-induced arthropathies, and plaques. An imidazolium-based IL and four biocompatible choline amino acid-based ILs ([Ch][AA]-ILs) were synthesized, which are 1-methyl-3-butyl-methylimidazolium iodide (BMIM-I), [Ch][Ala], [Ch][Ser], [Ch][Cys], and [Ch][His], respectively. The ILs were subsequently characterized using TLC, IR, and NMR spectroscopies. The solubility of commercial cholesterol was determined in the synthesized ILs by measuring fluorescence using a quantitative enzyme-based assay. The solubility of cholesterol in [Ch][Ser], [Ch][Cys], and [Ch][Ala] was determined to be 12.5 ±1.95, 136.3 ±42.3, and 4.95 ±0.855 ug/mL, respectively. The assay results show indiscernible solubility in BMIM-I and [Ch][His]. This suggests that the thiol group on [Ch][Cys] plays an important role in enhancing the solubility of cholesterol in that IL. This is a promising prospect in the treatment of atherosclerosis, and a potential treatment avenue for other diseases that are characterized by the formation of depositions or plaque. Lastly, these ILs can be integrated as a part of a more complex theragnostic drug delivery system, aiding in better understanding diseases while treating them. This project was supported by the BSU Department of Chemical Sciences, and by a summer 2022 grant from the BSU Adrian Tinsley Program.
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1. Introduction.

1.1. Discovery and Applications of Ionic Liquids

Ionic liquids are ionic compounds that have low melting points, making them liquid at room temperature, a very unusual property for ionic compounds. The earliest reported ionic liquid (IL), ethylammonium nitrate, [EtNH$_3$][NO$_3$], which has a melting point of 12°C, was discovered in 1914 by Paul Walden, although the potential of that discovery went unnoticed [1]. Decades later, ILs were “rediscovered” and noted in the literature by several researchers, such as Hurley and Weir in 1951 [1] and George Parshall in 1972 [1], each of whom studied them for different applications. However, it was not until the 1980s that they received widespread attention as a non-aqueous class of organic solvents due to their versatility [1]. In 1981, [EtNH$_3$][NO$_3$] gained a newfound interest, where its solvent properties were studied to be used in biochemical systems, which led to its study as an enzymatic catalyst at low concentrations in 1984 [18]. Moreover, [EtNH$_3$][NO$_3$] was also studied as a potential stationary phase in liquid chromatography, which opened the door for many other ILs to be used in that application [19]. Since then, different ILs have found their way into a variety of disciplines, including various fields of chemistry, medicine, pharmaceuticals, chemical engineering, environmental science, and even materials science [20].

Later on, the idea of using ILs as “designer solvents” caught the attention of many, as the wide variety of cation and anion combinations allows the solvent properties to be tuned as desired. From there, Task Specific ILs were introduced in the early 2000s by Jim Davis [19], which were similar to “designer solvents,” but contained a covalently bound functional group on the cation and/or the anion to guide its interactions with the desired substance. This versatility and inherent tunability of their properties made them a center of interest for a variety of applications, and they
grew to be used in the oil refining industry\textsuperscript{[20]}, carbon capture\textsuperscript{[12]}, catalysis\textsuperscript{[19]}, energy storage and as electrolyte materials\textsuperscript{[20]}.

\textbf{1.2. Biocompatible Ionic Liquids}

After the widespread attention that ILs received, it was only a matter of time before biocompatible ILs were designed. Biocompatible ILs are a class of ILs, often made as Task Specific ILs, that are created from naturally or biologically occurring compounds and their derivatives. One cation that has received a lot of interest recently for use in biocompatible ILs is choline, \((\text{CH}_3)_3\text{N(\text{CH}_2)_2\text{OH}}\). Choline is an essential nutrient that is both produced in the human body, and also obtained dietarily. Choline (used as choline hydroxide or choline chloride) is often paired with amino acids to produce choline amino acid ILs ([Ch][AA]-ILs), although it is also commonly paired with carboxylic acids or geranic acid (Figure 1) (CAGE) for biomedical uses. Since geranic acid is a metabolite that is used as an antifungal by many plants\textsuperscript{[22]}, CAGE is used as an antimicrobial IL. Moreover, amino acids are often paired with other cations than choline to create AA-ILs. Figure 2 shows the classification of recent biocompatible ILs in the literature\textsuperscript{[21]}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{(a) Structure of geranate, the conjugate base of geranic acid. (b) The structure of [EtNH$_3$][NO$_3$], the first reported IL.}
\end{figure}
1.3. Medical Applications of Ionic Liquids

A big, and relatively new, application of ILs is in the biomedical and pharmaceutical field, combating many rising medical crises. ILs can be used as therapeutic agents themselves, or as means to enhance existing treatments, such as drug delivery agents.

1.3.1. ILs as Drug Delivery Agents

ILs have been extensively researched as novel drug delivery systems, either as drug solvents \cite{7,8}, as active pharmaceutical ingredients (APIs) \cite{9}, or as a targeted drug therapy method, ancillary to the use of nanoparticles \cite{10}. For example, Banerjee et. al. \cite{11} have been using ILs to develop oral insulin, which could considerably raise patient compliance, lower the risk of dosing errors, while simultaneously reducing its cost. Moshikur et. al \cite{6} have also explored converting an anti-cancer active pharmaceutical ingredient, methotrexate, into an IL, effectively forming a cancer prodrug whose solubility is “5000 times higher than that of free [methotrexate] and two orders of magnitude higher compared with that of a sodium salt… [A] proline ethyl ester [methotrexate] prodrug showed similar solubility as the [methotrexate] sodium salt but it provided improved in

\textbf{Figure 2.} Composition of biocompatible ILs mentioned in the literature between 2020 and 2022. Tzani et. al, 2022\cite{21}.
Moreover, ILs have been used to change the administration route of a drug to avoid unpleasant side effects. Dinh et al.\textsuperscript{[14]} formed an IL by dissolving donepezil, a drug used to treat Alzheimer’s Disease, and dicarboxylic acid in ethanol. They then transformed the ILs into a transdermal patch, which showed improved skin permeability compared to that of free donepezil patches. By doing that, they eliminated the gastrointestinal side effects of donepezil, increasing patient compliance. Lastly, ILs have been used to enhance the intestinal absorption of drugs, overcoming the challenges posed by the GI tract on drug absorption\textsuperscript{[15]}.

1.3.2. ILs as Antimicrobial Agents

Antibiotic resistance is a prominent rising medical crisis, resulting in the death of at least 1.27 million people worldwide. The CDC reports more than 2.8 million antimicrobial resistant infections every year,\textsuperscript{[16]} making this an urgent and dangerous problem. In recent years, ILs have been investigated as new antibacterial agents\textsuperscript{[2,3]}, which could slow down the antibiotic resistance epidemic, and prevent the emergence of “superbugs.” Choline and Geranate (CAGE) based ILs have shown great promise as antimicrobial agents, effective against a myriad of bacterial, fungal, and viral species, while showing no toxicity to humans.\textsuperscript{[17]} A study by Miskiewicz et. al\textsuperscript{[3]} investigates the use of ILs to combat hospital-based infections, specifically \textit{Klebsiella pneumoniae, Acinetobacter baumannii, and Enterococcus} infections, which commonly originate in ICUs. These ILs can be delivered topically, intravenously, or orally, which makes them effective against a wide variety of infections.
1.3.3. **ILs in Cancer Treatment**

Cancer is one of the leading causes of death world-wide, and subsequently, there is a constant emphasis on cancer research and treatment. ILs have been employed as a new avenue of cancer research, not only for their drug enhancement and delivery properties, but also for their newfound antitumor properties \cite{5,6}. A 2015 study by Ferraz et. al \cite{18} showed the antiproliferative activity of ILs against five different cancer cell lines. The ILs in that study used ampicillin as an anion, and alternated between four cations (ammonium, imidazolium, phosphonium, and pyridinium). Moreover, those ILs presented no toxicity to normal human cell lines.

1.3.4. **ILs in Amyloidosis Treatment**

Many diseases, such as Alzheimer’s and Parkinson’s, are characterized by the aggregation of misfolded or mutated proteins, as the hydrophobic amino acid residues are exposed in an aqueous environment, causing the proteins to aggregate. The amyloid formation process can augment the progression of the disease and its symptoms. However, because of their tunable properties, ILs can provide a biocompatible solvent to dissolve these plaques and depositions, alleviating the symptoms and slowing progression of the disease. In amyloidogenic diseases, ILs have been observed to not only dissolve the plaque, but also restore the protein’s original function by refolding the proteins, completely reversing the plaque formation process, \cite{4} as shown in Figure 3 below:
Moreover, a 2009 study by Kalhor et al. tested the effect of several ILs, all of which used tetramethylguanidinium as a cation, on the amyloid formation of hen egg white lysozyme, and found that tetramethylguanidinium acetate inhibited in vitro formation of the amyloid by 50% [5].

1.4. Current Treatments of Atherosclerosis and Kidney Stones

Atherosclerosis and kidney stones are both diseases characterized by the formation of non-amyloidogenic aggregates. Atherosclerosis is a disease that is formed by the buildup of plaque in the inner lining of arteries, and can block the flow of blood and oxygen to the heart, causing heart attacks or even strokes. The plaque is mainly composed of cholesterol, although it also contains fibrin, cellular waste, calcium, and other fatty substances, all of which are insoluble, and thus aggregate in the arteries. At its earlier stages, atherosclerosis can be managed by dietary and lifestyle changes. As the disease progresses, a need for lifelong maintenance medications, specifically statins, arises. In more severe cases, such as plaque rupture or complete blockage of the arteries, surgical intervention is necessary [24, 25].
Kidney stones, on the other hand, are mainly composed of hard deposits of minerals, mainly formed of calcium oxalate monohydrate crystals. Besides being incredibly painful, kidney stones can cause permanent kidney damage, and increase the risk of urinary and kidney infections. Kidney stones are treated based on their severity, with less severe cases treated using pain management while the stones naturally pass, or using drugs to relax muscles in the ureter to help the stones pass quicker. Another treatment option that is used for larger stones is extracorporeal shock wave lithotripsy (ESWL) \(^{[23]}\), which uses sound waves to break up the stones into smaller pieces that can pass through the urinary tract. The next line of treatment, if ESWL doesn’t work, is percutaneous nephrolithotomy, which is a procedure that surgically removes kidney stones. While many treatment options for these diseases exist, these treatments are not ideal, as they carry many potential risks, such as infection, hemorrhaging, strokes, or other side effects of surgery. They also require patient compliance (for maintenance medications) and could be expensive. For those reasons, more affordable, less invasive treatments are needed \(^{[26]}\).

1.5. Scope of this Project

The aim of this project was to conduct a preliminary exploration of the use of ILs to dissolve arterial plaque. Different types of ILs were synthesized for testing. An imidazolium-based IL, 1-methyl-3-butyl-methylimidazolium iodide (BMIM-I) was synthesized for ease of synthesis, characterization, and examination, while choline amino acid-based ILs ([Ch][AA]-ILs) were synthesized due to their biocompatibility \(^{[13]}\), and are known to have high percent yields (>90%). The [Ch][AA] ILs synthesized were: [Ch][Ala], [Ch][Ser], [Ch][Cys], and [Ch][His], the structures of which are shown in Figure 4(a-e). The solubility of cholesterol (Figure 4f) was measured in the synthesized ILs using a quantitative enzyme assay, to determine which ILs can best dissolve cholesterol, which will indicate which ILs could be used to treat atherosclerosis.
Figure 4. (a) The structure of choline ([Ch]), the cation used in this project. (b) Structure of alanine. (c) Structure of serine. (d) Structure of histidine. (e) Structure of cysteine. The four amino acids ([AA]) were used as the anions. (f) The structure of cholesterol, the molecule of interest.
1.6. Significance of this Work

This project provides a new potential, less invasive, and less expensive approach for the treatment of diseases characterized by the formation of plaque or depositions.

1.6.1. Connection to UN SDGs

This project is directly connected to the UN SDG #3, Good Health and Well-being, by providing a novel class of potential therapeutics for diseases that affect many people worldwide. Moreover, these ILs can be used as a preventive measure, which would increase global health by preventing illnesses in the first place.

This project also indirectly ties to UN SDGs #6 and #12, which are Clean Water and Sanitation, and Responsible Consumption and Production, respectively, since this project could significantly decrease the need for maintenance medications, a lot of which ends up in the water, and thus decrease the amount of medical waste produced.

1.6.2. Applications in Human Space Flight

Because of their versatility and wide applications, ILs can be used as multi-purpose reagents in human space flight, to save space and weight. In space flight, ILs can be used for carbon capture, and as therapeutic agents. Moreover, since the gravitational change in space travel alters microbial physiology, essentially creating “superbugs” that are resistant to available antibiotics, ILs can be studied as antimicrobial agents to treat these infections.
2. Materials and Methods

2.1. Chemicals and Instrumentation

All chemicals were purchased from Sigma-Aldrich and used without further purification. 99% L-Alanine, Serine, Cysteine, and Histidine were used. 99.9% purity, HPLC grade methanol and acetonitrile were used. The cholesterol quantitation kit was also purchased from Sigma-Aldrich. Deionized water was used for all experimental work. To confirm the structure of products, Nuclear Magnetic Resonance (1H NMR) was performed using a JEOL ECX-400M spectrometer. All NMR samples were prepared in D$_2$O solvent. Fourier Transform Infrared (FTIR) spectroscopy was conducted using a Perkin Elmer Spectrum Two ATR/IR spectrometer. Fluorescence cholesterol assays were conducted using a Spectra Max Gemini EM plate reader.

2.2. Synthesis of 1-butyl-2-methylimidazolium iodide, BMIM-I

To prepare BMIM-I, 1.80g 1-methylimidazole (0.022 mol) were added to 3.5g 1-iodobutane (0.019 mol) in a 5-mL round bottom flask. An air condenser was attached to the setup after adding 1mL acetonitrile, and the reaction was left vigorously stirring for 90 minutes at 65°C. At the end of the reaction period, the vial was left to cool to room temperature before removing the acetonitrile under reduced pressure at 50°C using the rotary evaporator (rotavap). The solution was then purified using 3 rounds of liquid-liquid extraction with ethyl acetate, to remove the excess 1-methylimidazole. Lastly, excess ethyl acetate was removed using a rotavap, and the product was characterized using TLC, IR and NMR. BMIM-I was stored at 5°C until ready for use.
2.3. Synthesis of [Ch][AA]-ILs

All four [Ch][AA]-ILs were synthesized using the same procedure. 13.5mL (0.055 mol) of 46wt% choline hydroxide ([Ch][OH]), were added dropwise with stirring to 0.06 moles of aqueous amino acid solution (0.5 M Ser, 1 M Ala, 0.2 M Cys, 0.3 His) at 5°C. The reaction was left stirring overnight, in the dark, at 5°C. Afterwards, excess water was removed under reduced pressure using the rotavap at 50°C, and 25mL of 9:1 acetonitrile:methanol was added to precipitate out the excess amino acid, and that mixture was left stirring overnight at room temperature. Excess amino acid precipitate was removed using gravity filtration (Whatman #2 pleated filter paper), and the excess acetonitrile and methanol were removed under reduced pressure using a rotavap. The product was finally dried under vacuum for 24 hours. ILs were characterized using NMR and IR.

2.4. Characterization of ILs

$^1$H NMR was used to determine the structure and purity of the product, evaluating the accuracy of the synthesis. For all [Ch][AA]-ILs, 6-10mg of the pure product was dissolved in 0.8mL D$_2$O. For BMIM-I, 6mg of the pure product was dissolved in 0.8mL deuterated DMSO. IR spectroscopy was used to identify the structure of the chemical species, supplementary to NMR. UV-vis was used to confirm that the [CH][AA]-ILs do not absorb light at the wavelength used in the fluorescence assay, 535 – 587 nm. 10-15mg of the ILs were dissolved in 2mL H$_2$O, and the analysis was done using quartz cuvettes.
2.5. Cholesterol Assay

The cholesterol quantitation kit contained a buffer, an enzyme mix, a probe, and a cholesterol standard, which contained free cholesterol esters. Since the cholesterol added to the ILs for the solubility tests does not contain free cholesterol esters, they were not used, and a new standard was created instead by dissolving cholesterol in DMSO (2ug/mL). The standard was diluted to 25ng/mL before use, to stay within the linear range of the fluorescence assay. The standards contained 0, 0.1, 0.2, 0.3, 0.4, and 0.5 µg of cholesterol, and the total volume of each well plate was 100µL, including 2µL each of the probe and the enzyme mix.

To measure the solubility of cholesterol in the ILs, an excess (10 mg) of cholesterol was added to a 2:5 w/w IL:H₂O mixture in a 20 mL vial. The ILs were diluted, as they are viscous in their nature, to simulate the fluidity of intravenous drugs, since this project is aimed towards a medical application. The mixtures were left shaking at 200 rpm at 37 °C for 72 h before the assay was performed.

After 72 h, the [Ch][AA]-ILs were diluted with the buffer included in the assay kit to a total volume of 750 µL, and then centrifuged for 15 min. A fraction of the supernatant was diluted again in the buffer to a total volume of 200 µL, and then filtered using 0.45 µm filters, before using in the assay. After trial and much tribulation, the dilution factors (DF) that yield results within the linear range were found, and are outlined in Table 1. Diluting the sample was necessary as the ILs had a yellow color, which may interfere with the fluorescence of the enzymatic reaction. [Ch][Cys] had the most intense color, and so it was diluted the most. BMIM-I did not need further dilution after the 2:5 mixture, as it was a clear, colorless liquid, although it did follow the same purification method (centrifugation and filtration). Each sample was run in triplicate.
To each well, 5 or 10 µL of the diluted, purified sample was added to the well plate, with 2 µL each of the probe and buffer, and 81 or 86 µL of the buffer. The samples were left to incubate, protected from light, at 37 °C for 1 h to allow the enzymatic reaction to go to completion, which is what creates the fluorescence. The plate was then set to shake for 5 s after the 1 h incubation period, before obtaining fluorescence readings.

Table 1. Dilution Factors of [Ch][AA]-ILs Before Use in the Essay

<table>
<thead>
<tr>
<th>IL</th>
<th>IL:H₂O mixture (µL)</th>
<th>Buffer (µL)</th>
<th>DF #1</th>
<th>Supernatant (µL)</th>
<th>Buffer (µL)</th>
<th>DF #2</th>
<th>Sample (µL)</th>
<th>Probe, Buffer, Enzyme mix (µL)</th>
<th>DF #3</th>
<th>Total DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ch][Ala]</td>
<td>500</td>
<td>250</td>
<td>1.5</td>
<td>100</td>
<td>100</td>
<td>2</td>
<td>10</td>
<td>90</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>[Ch][Ser]</td>
<td>250</td>
<td>500</td>
<td>3</td>
<td>100</td>
<td>100</td>
<td>2</td>
<td>10</td>
<td>90</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>[Ch][Cys]</td>
<td>175</td>
<td>575</td>
<td>4.3</td>
<td>25</td>
<td>175</td>
<td>8</td>
<td>5</td>
<td>95</td>
<td>20</td>
<td>688</td>
</tr>
<tr>
<td>[Ch][His]</td>
<td>500</td>
<td>250</td>
<td>1.5</td>
<td>100</td>
<td>100</td>
<td>2</td>
<td>10</td>
<td>90</td>
<td>10</td>
<td>30</td>
</tr>
</tbody>
</table>
2.6. Fluorescence Plate Reader Settings

Since the fluorescence is linearly proportional to the amount of cholesterol present, a fluorescence microplate reader was used to quantify the amount of cholesterol dissolved in each sample. The software used was Softmax Pro 6.4 application, which allows for temperature control, and thus the samples were left to incubate at 37 °C in the microplate reader. From the
Template Editor, the wells containing the blank, standards, and unknown samples were selected and named accordingly. The concentration unit of the standards was changed from the Edit Standards menu under Template Editor. Afterwards, read mode was selected as Fluorescence from the Settings menu, and read type was selected as Well Scan. The excitation wavelength was set to 535nm, and the emission wavelength was set to 587nm. The plate type was set as a 96-well plate, and the read area was selected as the wells containing samples. The samples were set to shake for 5 seconds before reading. Lastly, from the Display menu from the Home Screen, Raw Data with Reduced Number was selected. The software interface is shown in Figure 9 below.
Figure 6. Softmax Pro Software Interface
3. Results and Discussion

Figure 7. Synthesized [Ch][AA]-ILs, stored at 5°C. [Ch][Ala], [Ch][His], and [Ch][Ser] have a similar yellow color, but [Ch][Cys] has a darker, orange color.

Figure 8. [Ch][AA]-ILs after being left at room temperature for 9 months. [Ch][Ser] changed from a light yellow to a deep orange, and [Ch][His] changed to a slightly orange color. [Ch][Cys] darkened slightly, and [Ch][Ala] did not change.

$^1$H NMR scans were repeated after 6 months of leaving the ILs at room temperature, and no structural changes were present.
3.1. Characterization of ILs

3.1.1. Characterization of BMIM-I

The structure and purity of BMIM-I was determined using TLC (Figure 9a), using a developing solvent of 8:1.5:0.5 ethyl acetate:ethanol:ammonium hydroxide. The TLC was used to ensure that the excess reagent, 1-methylimidazole was adequately removed. IR (Figure 9b), and 1H NMR (Figure 9c) were used to ensure the structure of BMIM-I, and that no side products or contamination is present.

![TLC Plates](image1)

![IR Spectrum](image2)

**Figure 9.** (a) TLC Plates to detect any impurities in BMIM-I. The sample on the left lane is 1-methylimidazole, the excess reagent, visualized using iodine staining. The sample on the right is purified BMIM-I product. The TLC plate indicates no impurity in the form of excess reagent. (b) IR spectrum of synthesized BMIM-I shows some ethyl acetate contamination.

The ethyl acetate contamination indicates that there may be some limited solubility of ethyl acetate in BMIM-I, since it was not fully removed under reduced pressure using the rotavap.
All [Ch][AA]-ILs were characterized using 1H NMR spectroscopy, dissolved in D$_2$O, and the results were compared to the spectra reported in the literature. The ILs were also characterized using IR spectroscopy, to further confirm the structure. The obtained $^1$H NMR spectra (figures 10—13) were compared to the those reported in the literature, and the results were consistent, confirming the structure and purity of the synthesized ILs, thus indicating the accuracy of the synthesis. In [Ch][Ser] and [Ch][His], the signal adjacent to the chiral center on the amino acid, which is theoretically a doublet in both, showed up as a symmetrical double doublet (DD) in both. This is consistent with the results reported in the literature, and the symmetry of the DD
signal indicates that it may be due to the non-superimposable diastereomer, although more research is needed to confirm that theory.

**[Ch][Ala] Characterization**

**Figure 10.** $^1$H NMR spectrum of synthesized [Ch][Ala] matches what is reported in the literature. Signals B and C are, theoretically, doublets, but show as multiplets on the spectrum. This matches the results reported in the literature.
Figure 11. $^1$H NMR Spectrum of [Ch][Ser] matches what was reported in the literature. Signals B and C are multiplets as well. Signal D (–CH$_2$) is a symmetrical double doublet, which is most likely due to the chiral center adjacent to it, but more research is needed to confirm that.
Figure 12. NMR spectrum of [Ch][His] matches what was reported in the literature, confirming the purity and structure of the product.
Figure 13. $^1$H NMR spectrum of [Ch][His] matches what was reported in the literature. Signal D is a double doublet.
3.2. Fluorescence Assay Standards and Samples

Of all the samples that were run to determine the dilution factor for each IL, Figures 14 and 15 below show the two standard curves of the trials that were used to perform the solubility calculations. The total cholesterol content, in µg, in each well was determined using the standard calibration curve. The solubility of cholesterol in each IL, expressed in µg/mL, was determined from the total cholesterol content (multiplied by the DF), divided by the original volume of IL.

**Figure 14.** Standard curve used to calculate the solubility of cholesterol in [Ch][Ala] and [Ch][His]. The R² value of 0.995 points towards the accuracy of the data.

**Figure 15.** Standard curve used to calculate the solubility of cholesterol in [Ch][Cys] and [Ch][Ser]. The R² value of 0.9983 points towards the accuracy of the data.
3.3. Total Cholesterol Content in ILs

Table 3. Cholesterol Solubility Data of [Ch][AA]-ILs, extracted from two standard curves.

<table>
<thead>
<tr>
<th>IL</th>
<th>Absorbance</th>
<th>Average Cholesterol Content in Well Plate (µg)</th>
<th>DF</th>
<th>Average Cholesterol Content in Initial 750ug Vial (µg)</th>
<th>Solubility of Cholesterol in IL (µg/mL)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ch][Ser]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>1450.</td>
<td>0.1418</td>
<td>60</td>
<td>8.505</td>
<td>11.34</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>1553</td>
<td>0.1518</td>
<td>60</td>
<td>9.111</td>
<td>12.15</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>1925</td>
<td>0.1882</td>
<td>60</td>
<td>11.29</td>
<td>15.05</td>
<td></td>
</tr>
<tr>
<td>Avg</td>
<td>1643</td>
<td>0.1606</td>
<td>60</td>
<td>9.635</td>
<td>12.85</td>
<td>1.953</td>
</tr>
<tr>
<td>[Ch][Cys]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>981.3</td>
<td>0.09594</td>
<td>688</td>
<td>66.01</td>
<td>88.01</td>
<td></td>
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<tr>
<td>T2</td>
<td>1858</td>
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<tr>
<td>[Ch][Ala]</td>
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<tr>
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<td>BMIM-I</td>
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The solubility of cholesterol in [Ch][His] and BMIM-I was constantly below the limit of detection.
4. Conclusions

Since their discovery, biocompatible ILs have been implemented in a variety of applications, combating many rising medical crises. This project studied the solubility of cholesterol in [Ch][AA]-ILs, as an investigation into their application to treat atherosclerosis. After visualizing and localizing arterial plaque using imaging techniques, such as MRI or ultrasound, the proposed treatment includes using the diluted ILs as an intravenous injection, as a part of a nanoparticle drug delivery system, targeting the plaque itself. Moreover, to prevent the breakoff of blood clots, which could cause strokes, the nanoparticle could be laced with a blood thinning agent, such as heparin. After treatment, imaging techniques would be used to measure the size of the remaining plaque, if any remains. Prevention of recurrence of plaque is dependent on patient compliance with medical recommendations on diet and lifestyle.

The results show that, out the five synthesized ILs, [Ch][Cys] is the best solvent for cholesterol. In fact, [Ch][Cys] shows a 10-fold increase in solubility than the next best solvent, [Ch][Ser], despite the similarities in their structures. BMIM-I and [Ch][His], both of which contain an imidazole ring, both did not show any discernible solubility, which points towards the fact that imidazole groups do not function as proper solvents for cholesterol.

![Figure 16. Side-by-side structures of Cysteine (left) and Serine (right). The two amino acids are identical except for a hydroxide group on the serine instead of the thiol group on the cysteine.](image)
Since sulfur and oxygen have very similar chemical properties, a 10-fold difference in solubility is surprising. It is clear that the thiol group somehow enhances solubility, possibly by forming disulfide bonds. Cysteine is a highly active amino acid in biological systems, and the thiol group is readily deprotonated due to its pKa of 8.5, forming cystine in solution (R—S—S—R) in solution. Moreover, the thiol group is a good nucleophile, as opposed to the hydroxide group on serine, and it is possible that it undergoes an SN2 reaction with the choline (a good leaving group), to form a complex of the structure RS—C—C—OH, which could be responsible for the enhanced solubility. Further investigation into the role of disulfide bridges, or more specifically thiol groups, on enhancing the solubility of ILs is needed. This can be done by using other thiol-containing anions instead of cysteine, and analyzing the effect they have on ILs’ solubility. The role of cysteine in enhancing solubility can also be investigated by using cysteine-based ILs and varying the cation.

Alternatively, this project can be advanced by studying the ability of these ILs to dissolve calcium oxalate, the main component of kidney stones, to provide a less painful, less invasive, and less expensive treatment for larger kidney stones. Moreover, the ability of these ILs to prevent cholesterol from aggregating in the first place, or kidney stones from forming, can be studied. That would allow for their use as preventive measures, as opposed to treatment agents. Lastly, this project can be integrated as a part of a more complex theranostic nanoparticle drug delivery system, carrying the therapeutic agent and a contrast agent, allowing for real-time adjustment of the treatment based on patients’ needs. Theranostics are a relatively new avenue of medicine that is derived from combining therapeutics with diagnostics [27], allowing for more patient-specific treatments.
To study the ability of ILs to dissolve kidney stones, calcium oxalate monohydrate can be synthesized following procedures outlined in the literature [28]. Afterwards, an oxalate quantification kit (available at Sigma Aldrich) can be used to measure the solubility of calcium oxalate in the ILs in a similar fashion to this study. Afterwards, the ability of these ILs to prevent calcium oxalate monohydrate from forming can be investigated by using a water:IL matrix in the synthesis process, and studying the results.
5. Acknowledgements

While I cannot begin to acknowledge all the people that have supported me throughout, shaped my perspective, and helped me become the person I am today, there are some people to whom I am always grateful.

First, I would like to thank my mentor, Dr. Edward Brush, for the invaluable help, support, and guidance he has given me. I would like to thank him for his endless patience and inspiration, and for encouraging me to explore in research and find what I’m truly passionate about. I would also like to thank him for everything he’s taught me, in lab and in life. This project would not have been a reality if it wasn’t for his help and support.

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This thesis is being submitted in partial fulfillment of a Bachelor of Science degree with Commonwealth Honors in Chemistry (Professional Chemistry concentration).
6. References


