Ionic liquids and antibiotics- do they work together?

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Summary and research aims:

Ionic liquids (ILs) in the recent years have had an extremely popular role in the scientific industry, where due to their unique properties, they have been found to have variable applications in many areas of modern sciences, and in medical fields such as drug delivery and presenting anti-bacterial properties . A recent issue has been overcoming skin-based bacterial biofilms, which have become rapidly resistant to a wide range of antibiotics including Penicillin, posing a huge risk to the development of chronic diseases within vulnerable patients. However, scientists have found that ILs could be beneficial in overcoming bacterial biofilms through disrupting the bacteria's growth and so therefore preventing its spread.

This research poster will explore the concept of whether ionic liquids could be used in antibiotics through experimenting whether they would be beneficial in treating bacterial biofilms or not.

What is an ionic liquid and why is it important?

Ionic liquids (as shown in **figure 1**) are ionic compounds (or molten salts) that are at approximately room temperature, due to their irregular structure and unsymmetrical ions, abandoning the usual large ionic lattice structure of basic ionic compounds . A common example of a basic ionic compound is table salt (NaCl), where its basic structure is shown in **Figure 2**. Ionic liquids consist of both positively and negatively charged ions (anions and cations) where examples of structures of anions and cations are shown in **figure 3** below.

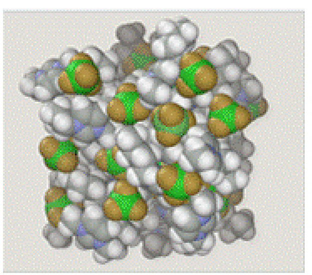

Figure 1. Proposed structure of an imidazolium-based ionic liquid.

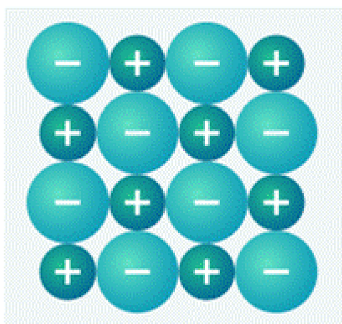

Figure 2. 2D structure of a sodium chloride lattice.

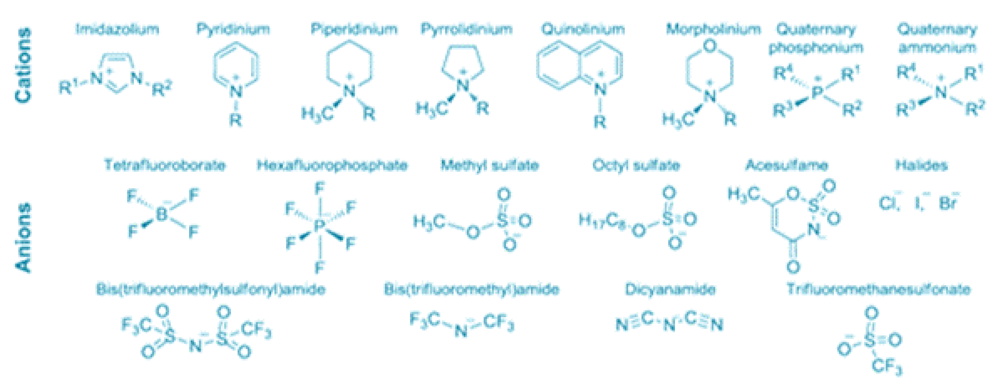

Figure 3. Examples of cations and anions commonly used in ionic liquids

What are the properties of ionic liquids?

Ionic liquids are known to have non volatile and non flammable properties, which make them environmentally safe, and so therefore referred to as "green" chemicals as they are safer than the majority of other solvents or chemicals used in science . Furthermore, the majority of ionic liquids are soluble, allowing them to dissolve many substances that traditional solvents cannot, however, for non soluble ionic liquids, its chemical properties could be altered due to their tunable nature – whether that's changing the chain length or altering the composition of the ionic liquid (such as the hydrophilic/hydrophobic properties) so they can possess specific characteristics.

What are the biological applications of ILs?

The discovery of some safe and non-toxic ionic liquids has had a major role in its use in living organisms, where researches have concluded that the longer the alkyl chain length, the higher the toxicity of the ionic liquids, and that many amino acid and Choline based ionic liquids have been found to have a lower toxicity. Furthermore, (as shown in **Figure 4**) increasing the alkyl chain length increases the anti-bacterial efficacy of ILs, as long alkyl chains destabilize the bacteria cell membrane, enabling the alkyl chain to penetrate the phospholipid bilayer, resulting in structural damage to the bacterial cell membrane, resulting in cell lysis, so the bacterial cells can no longer divide or multiply.

As seen in **Figure 5**, this gives ionic liquids a high biological activity (capable of achieving biological effects); meaning that they could be used in fields such as drug synthesis , drug delivery, ionic liquid drugs (using traditional drugs in the form of ionic liquids species) biomedical analytics (such as chromatography, cancer biomarkers or the detection of natural and pharmaceutical substances) in addition to Ioliomics (which is the study of ions in liquids and revealing the fundamental properties of ionic interactions in chemical and biological systems.

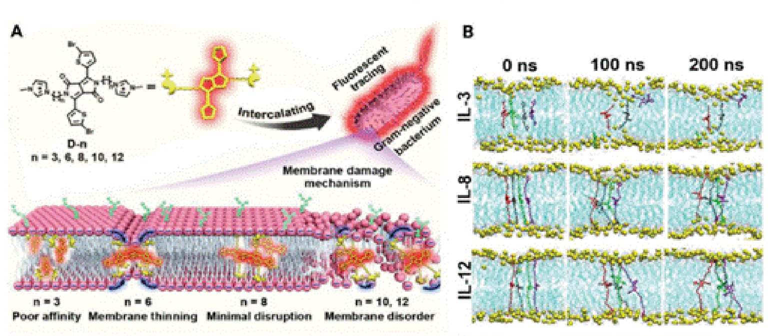

Figure 4. Effect of alkyl chain length on antibacterial activity of ionic liquids. A) Schematic of membrane damage by a series of ILs with different alkyl chain on bacteria. B) Sequential snapshots of interactions between ILs and the phospholipid bilayer.

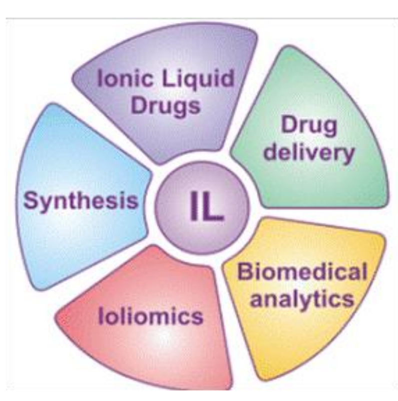

Figure 5. Applications of ionic liquids (IL) in pharmaceutics and medicine

ILs and the disruption of skin- based bacterial biofilms?

Skin-based bacterial biofilms are clusters of bacteria which form on the surface of the skin, containing substances such as DNA, proteins and polysaccharides, allowing the bacteria to resist antibiotics or any defence host cells. A main example of a bacteria present in the biofilm includes Pseudomonas Aeruginosa, which is shown to be able to grow on non-living surfaces such as hospitals. However, it has been discovered that an ionic liquid called Choline Geranate which is seen as beneficial in disrupting the biofilms and offering minimal toxicity towards the skin and epithelial cells.

Materials and proposed method:

Figure 6. A culture dish with Pseudomonas Aeruginosa

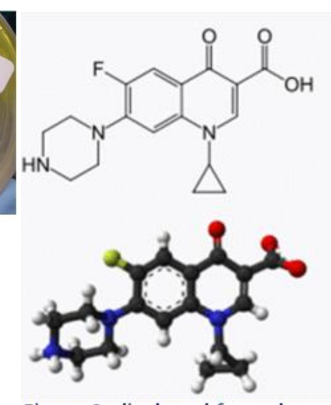

Figure 8. displayed formula and 3D structure of Ciprofloxacin

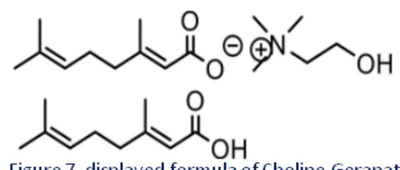

Figure 7. displayed formula of Choline Geranate

Apparatus and materials used:

- Pseudomonas Aeruginosa frozen stock
- LB Agar
- Ciprofloxacin 0.25 µg/ml antibiotic suspension
- Choline- Geranate ionic liquid
- Petri dishes
- Sterile wooden applicator sticks
- Incubator
- Autoclave machine

Proposed Method:

1. Place the LB Agar (pre-prepared) in an autoclave for sterilisation, then allow the LB Agar to cool down slightly.
2. Add the antibiotic (Ciprofloxacin 0.25 µg/ml) only in one LB Agar mixture and stir. Complete the same process by adding the Choline- Geranate ionic liquid in a second LB Agar mixture. Allow the agar mixtures to cool down at room temperature, then pour a suitable amount of each mixture into sterilised Petri dishes.
3. Scrape a small amount of bacteria from frozen stock. (Do not thaw frozen stock, scraping a small portion of frozen bacteria is sufficient, repeated freezing and thawing of stocks will result in reduced viability.)
4. Streak bacteria onto agar plate using sterile applicator sticks.

To ensure that the results obtained are valid, and that the outcome is due to the antibiotic and ionic liquid, many controls have been applied including :

- Sterilised LB Agar only
- Sterilised LB Agar + Pseudomonas Aeruginosa Bacteria
- Sterilised LB Agar + Ciprofloxacin 0.25 µg/ml
- Sterilised LB Agar + Choline- Geranate ionic liquid

5. Incubate all the previous agar plates at 37°C for 16–24 hours

Results:

After incubating the Petri dishes for 16-24 hours, they will be examined based on whether the bacteria continued to grow (and form colonies) to suggest that either the antibiotic or ionic liquids were ineffective, or if the bacteria doesn't grow which would mean that the antibiotic or ionic liquids were effective.

The expected results (for if I had completed the experiment myself) are written in the table below:

Experimental design	Observations	Comments based on observations made:
LB Agar alone	The agar remains clear	There is no contamination of the LB Agar
LB Agar + Pseudomonas Aeruginosa	The bacteria grows to form clear colonies	The bacteria added is not contaminated
LB Agar +Ciprofloxacin 0.25 µg/ml	The agar remains clear	There is no contamination of the antibiotic.
LB Agar +Choline- Geranate	The agar remains clear	There is no contamination of the ionic liquid.
LB Agar + Pseudomonas Aeruginosa + Ciprofloxacin 0.25 µg/ml	The agar should be clear	The bacteria is not resistant to the antibiotic given.
LB Agar + Pseudomonas Aeruginosa + Choline- Geranate	-	-

Analysis and conclusions:

Due to challenges faced by school due to COVID-19 restrictions and the inability to provide the key chemicals and materials in order to perform the experiment, this has prevented the ability to conduct the experiments desired. Therefore, I have left empty sections on my table in order to include the results obtained when I complete this experiment in the near future.

Based on the research I have found on ionic liquids and Choline-Geranate in particular, when adding the ionic liquid alone to the bacteria, this should result in a disruption in the growth of the bacteria, where there should be clear areas detected on the agar if not the whole agar, due to the Choline Geranate damaging the bacterial cell membrane resulting in the lysis (destruction) of the bacterial cells. If the ionic liquid manages to produce the same impact that the antibiotic has on the bacteria, this would mean that the ionic liquid is just as effective as the antibiotic and could therefore be used alongside or instead of antibiotic treatment and specifically for skin based bacterial biofilms.

However, in the case of testing if ionic liquids can be used alongside antibiotic treatment for skin based biofilms, this would require different experiments to explore whether the ionic liquid would have a positive or negative effect on the efficacy of the antibiotic or whether it allow the antibiotic to penetrate any skin barriers allowing the antibiotic to become more effective on disrupting the bacterial growth and spread.

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