Ionic Liquids
Investigating Antimicrobial ability

King Edward VI Camp Hill School for Boys

Hassan, Oliver, Omar, Anthony

Introduction

Ionic liquids are a type of liquid consisting entirely of ions (charged particles) instead of neutral molecules at room temperature.

They typically have low melting points, high boiling points, and low vapour pressure, making them relatively stable and easy to handle.

Our interest was in whether the ionic liquids we produced have any apparent antibacterial properties.

Research aims

- To evaluate the potential of ionic liquids as alternative disinfectants to traditional chemical agents
- To investigate the mechanisms by which ionic liquids exert their antimicrobial effects, such as disruption of the cell membrane
- To explore the potential of using ionic liquids as surface coatings or additives to prevent the growth of microorganisms on various materials, including medical devices or food packaging.

Synthesis of the ionic liquids

Before synthesis began, we first determined what mass of haloalkane would be needed to produce BMIM Cl and BMIM Br, based on a starting mass of 5g MIM.

Two batches of BMIM Cl were prepared by refluxing the reactants for 2 hours, at which point we estimated that the reaction was complete. We also tried to produce a batch of BMIM Br, but it appeared to decompose on heating without a solvent.

Due to their viscosity, both batches of BMIM Cl were heated first and suspended upside down above the sample tube to be collected. Most of each sample was recovered, but some of our yield remained on the inside of the reaction flask.

With the batch of BMIM Br that decomposed, some effort was made to recover any undamaged IL by dissolving it in water and adding some $Al_2(SO_4)_3$ to act as a clumping agent to remove the dark solid which we believe was carbon. We weren't successful and so the BMIM Br batch was discarded.

The BMIM Cl batches (Batch 1 and Batch 2) were then taken into the next phase of the investigation.

Methylimidazole	**82.0 g/mol**	Batches					
mass:	4.999	Number	1	2	3	4	5
		Date	13/3/2023	13/3/2023	13/3/2023	20/3/2023	20/3/2023
mol:	6.10E-02	IL	BMIM Cl	BMIM Cl	BMIM Br	BMIM Br / 40mL water	BMIM Br / 40mL EtOH
		MIM mass	4.999	5.060	5.005	5.002	4.998
1-chlorobutane	**92.5 g/mol**	halo mass	5.682	5.712	8.364	8.357	8.346
mass:	5.639	yield	6.754	8.395	n/a	8.3883	13.477
		Note			Appeared to decompose. Attempts made to recover it by dissolving in water and filtration.		
1-bromobutane:	**136.9 g/mol**						
mass:	8.346						
		Batches					
potassium hexafluorophosphate	**184.1 g/mol**	Number	6	7	8	9	10
mass:	11.223	Date	27.3.23	27.3.23	27.4.23		
		IL	BMIM BF4	BMIM PF6	BMIM FeCl3Br		
potassium tetrafluoroborate	**125.9 g/mol**	MIM mass	5.002	4.999	1.876		
mass:	7.675	halo mass	5.655	5.648	3.132		
		-ve ion mass	7.672	11.233			
1,4-dichlorobutane	**127.0 g/mol**						
mol:	3.05E-02						
mass:	3.871						

Figure 1 – a copy of the synthesis log which we kept during the project

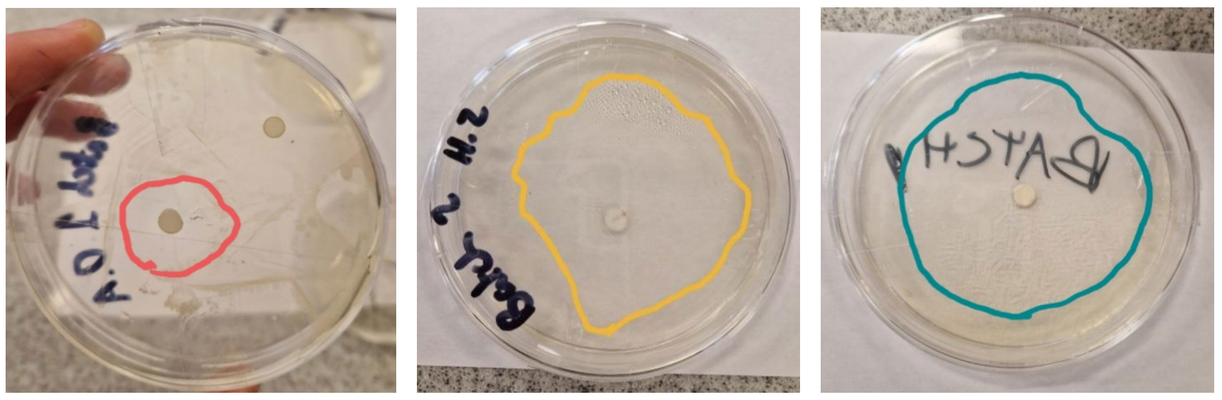
Our Investigation

We wanted to test whether our batches of BMIM Cl had any use as antimicrobial agents. To do this we needed to carry out an aseptic technique practical.

We used agar plates and a growth medium to culture E-coli for testing against our IL samples. Firstly we had to create a sterile environment. The workspace was cleaned with an antiseptic (Virkon) and the neck of the E-coli culture bottle was flamed to make sure no external bacteria would contaminate our sample.

A sample of the E-coli growth medium was transferred to the agar plate. The lid of the agar plate was only removed briefly to place the culture on the agar, before being put back on to prevent contamination. We then used a sterilised metal spreader to distribute the agar evenly around the plate, before applying small circles of filter paper soaked in our BMIM Cl to the culture and leaving it to incubate overnight at 25°C.

Results



BMIM Cl (Batch 1) plate

Small, clear zone of inhibition around the IL

BMIM Cl (Batch 2) plates

Larger zone of inhibition around the IL, but less clear than with Batch 1

Figure 2 – the agar plate experiments suggested the BMIM Cl was able to kill the strain of E-coli which we used

Analysis & conclusion

Due to the zones of inhibition present on the agar plates of both batches, we can see that BMIM chloride does appear to have antimicrobial properties, as seen by the E-coli death around the discs soaked in the ionic liquid.

We can see that Batch 1 has a stronger, smaller zone of inhibition than Batch 2, possibly caused by the differing viscosities of the two separate batches. Batch 2 might have been able to disperse throughout a greater area of agar since it appeared to be less viscous than Batch 1.

In order to test for a relationship between viscosity and efficacy we repeated the experiment for both batches using a 0.1% and 0.01% dilution with water. Neither batch at any dilution produced a zone of inhibition, so reducing the viscosity of the ILs to allow them to diffuse throughout the agar did not increase the zone of inhibition or any antimicrobial effect.

Perhaps in the future if we were to compare the zones of inhibition produced by other ILs we could better understand their antimicrobial properties, and determine which is the most effective.

Further investigation

We then moved to consider what the cause of BMIM Chloride's antimicrobial activity might be, and decided upon two possible reasons:

- The water potential of the BMIM Cl was lower than that of the E-coli, causing water to leave by osmosis resulting in plasmolysis
- The shape and polarity of the BMIM cation being similar to that of a phospholipid may have damaged or affected the cell membrane

While we didn't have the equipment to view the E-coli to determine if the cell membranes were being damaged by our ionic liquid, we could explore the effect of BMIM Cl on osmosis.

To determine if it had a low enough water potential to cause plasmolysis. We performed an experiment in which potato cubes of equal size were placed in equal volumes of Batch 1 and 2 as well as an 82% sugar solution. The sugar solution was meant to simulate another known antimicrobial: honey. After three hours the potato cubes were dried and measured and the results are below:

	Initial mass (g)	Final Mass (g)	Change in mass (g)	Percentage change
Batch 1	1.658	0.948	0.710	42.8%
Batch 2	1.341	0.762	0.579	43.2%
82% sugar solution	0.877	0.438	0.439	50.1%
control	1.084	0.922	0.162	14.9%

Figure 3 – the results from an experiment to investigate BMIM Cl's relative water potential

Both BMIM Cl batches showed a similar decreasing effect on the mass of the potato cubes, causing water to be pulled out of the potato cells leading to plasmolysis. From this, we can infer that BMIM Cl has some efficacy for inducing plasmolysis of the E-coli cells we studied, through causing the osmosis of water outside of the bacterial cells.

We saw that the Batch 1 BMIM Cl didn't inhibit the E-coli as well as Batch 2, probably due to a difference in viscosity. It was also noted that the sugar solution was also more effective at causing plasmolysis in the potato cells than either of our BMIM Cl batches.

In the future we could have investigated the osmotic potential of different BMIM compounds, and investigate how their charge to size ratios may have affected the rate of osmosis, making them a more effective antimicrobial.