International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Elucidation of the Toxicity of Imidazolium-based Surface-Active Ionic Liquids (Im-SAILs) against Gram-Positive and Gram-Negative Bacteria.

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Abstract

Imidazolium-based Surface-Active Ionic Liquids (Im-SAILs) are a type of surfactant that has gained significant attention in recent years due to their unique properties and potential applications in various fields. They are perceived to be less toxic than other synthetic solvents and are being explored for applications in various fields including biotechnology, chemical engineering, pharmaceutics, and many others. This manuscript investigates the bacterial toxicity of varied concentrations of Im-SAILs to Gram Positive and Negative bacteria. Results show that 1-dodecyl-3-methylimidazolium tetrafluoroborate posed a higher overall bacterial toxicity compared to 1-decyl-3-methylimidazolium tetrafluoroborate and 1-octyl-3-methylimidazolium tetrafluoroborate with corresponding disk diffusion analysis across various Gram-Negative and Gram-Positive bacteria. These results help establish the perceived safety of Im-SAILs as a choice surfactant for oil spill dispersion in marine ecosystems. Findings from this study will be beneficial to ascertain if this class of surfactant poses a threat to aquatic organisms and would encourage regulated or controlled use, as necessary. This would also ensure the protection of aquatic life and biodiversity as aligned with the United Nations Sustainable Development Goal 14.

Keywords: Imidazolium-based-surface-active Ionic Liquids, Disk Diffusion, Toxicity, UN SDG 14

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1. Introduction

Imidazolium-based Surface-Active Ionic Liquids (Im-SAILs) are a group of cationic surfactants that are commonly used as fabric softeners, textile auxiliaries, asphalt additives, corrosion inhibitors, oil spill surfactants and biocides. Over the past decades, ILs have been in the spotlight of the scientific and industrial community as a promising alternative to replace the traditional organic solvents. In addition to their unique ionic liquid (ILs) inherited properties and being less toxic than other synthetic solvents, their superior to conventional surfactant surface properties have opened new perspectives for application of SAIL based micellar systems in various fields including biotechnology, chemical engineering, pharmaceutics, and many others [1]. Working with SAILs has several benefits, including the ability to quickly modify the structural and functional properties of their cationic and anionic substituent groups to fine-tune their hydrophobic and hydrophilic properties [2]. However, studies assessing the toxic properties of ionic liquids (ILs) have been largely based on acute 'short-term' toxicity studies. The increased use and application of ILs will inadvertently lead to an increased and sustained release into the environment through direct and indirect sources. This would increase the likelihood of sustained exposure of organisms in such environments including marine ecosystems. There is a need to evaluate both short- and long-term effects of ILs to establish the perceived non-toxic properties of the liquids. Cationic surfactants account for almost 7 % of the total surfactant market [3]. Imidazolium-based surface- active ionic liquids (Im-SAILs) are cationic surfactants based on imidazolium salts with long hydrophobic alkyl chains. Im-SAILs exhibit novel aggregation behaviour that differs from that of conventional surfactants which makes them a choice candidate for oil recovery. The desirable features of IL-based surfactants as opposed to conventional surfactants include environmental friendliness, low toxicity, non-corrosive, negligible vapour pressure, high thermal stability, and are recyclable [4]. However, ILs also have remarkably high solubility in water and other solvents, they have been reported to be resistant to photodegradation and can also be bioaccumulated in biological tissues, these characteristics make them potentially toxic class of chemicals in aquatic ecosystems. Several studies have been conducted to assess the relative toxic potential of ILs, most of these studies were done using microorganisms and to assess short-term toxic effects only [5, 6, 7]. In this proposed study, we

aim to conduct interdisciplinary and collaborative research to assess the possible short - and long-term toxic effects of synthesised ILs. Since Im-SAILs are choice candidates for oil recovery, we will be assessing the possible toxic effects of the synthesised ILs on a marine species. This is to enable us to establish the relative safety of the use of ILs as oil dispersants in marine ecosystems.

2. Materials and methods

2.1. Materials

1-octyl-3-methylimidazolium tetrafluoroborate, 1-decyl-3-methylimidazolium tetrafluoroborate and 1-dodecyl-3-methylimidazolium tetrafluoroborate (OMIM BF_4, 99 %, C_{10}MIM BF_4, > 98 %, C_{12}MIM BF_4, > 98 %) was purchased from Fisher Scientific Sdn. Bhd.

2.2. Quantitative Assay

Ecotoxicity of Im-SAILs mixture was determined using broth microdilution method according to standard procedure CLSI-M07-A9, 2008, developed by Clinical and Laboratory Standard Institute (CLSI), USA. Im-SAILS solution was diluted using two-fold serial dilution and added into each of the 96 wells. IM-SAILS solutions were added accordingly into each well from 100,000 ppm (10 wt. %) to 200 ppm (0.020 wt. %). Bacterial suspensions *Staphylococcus aureus* (+), *Bacillus cereus* (+), *Candida albicans* (+), *Pseudomonas aeruginosa* (-) *Escherichia coli* (-) and *Aspergillus fumigatus* (-) was prepared by transferring a loopful of colony from 24-hour bacteria culture to Mueller Hinton broth. About 100 µL of each microorganism was added into each well containing 100 µL of IL mixture in separate microdilution plate. About 100 µL of microorganism suspension was used as blank and dispensed into the first well. Microdilution plates were incubated at 37℃ for 24 hours and absorbance value of each well was determined using microplate reader (Cyber ELISA-R01 Micro-platereader, Cyberlab) at a wavelength of 550 nm. The minimum inhibitory concentration (MIC) and EC50 values of Im-SAILS solution was determined by plotting the absorbance values in a graph generated by Prism version 9 (GraphPad).

2.3. Qualitative Assay

Disc diffusion method was carried out to verify the toxicity results obtained from broth microdilution assay. Approximately 100 µL of overnight microorganisms' culture was spread on Mueller Hinton agar. Blank antimicrobial disc was placed onto the centre of the bacteria suspension, followed by the inoculation of 25 µL of the respective Im-SAILs. The susceptibility of each Im-SAILs towards the microorganisms was conducted in triplicates with a positive control of only bacterial suspensions and negative control of sterile antimicrobial disc inoculated with Im-SAILs. The petri plates were incubated at 37 °C for 24 hours. The diameter (D) of inhibition zones will be measured and evaluated according to the scores developed by Jaganathan et. al. [8], in which 6 mm equals to no inhibition (-), D < 11 mm represents weak inhibition (+), 11 mm < D < 21 mm represents good inhibition (++) and D > 21 mm represents strong inhibition (+++).

3. Results and discussion

Table 1 presents the results of broth microdilution tests conducted on various microorganisms, revealing the ecotoxicity pattern of the three Im-SAILs. The potency of the Im-SAILs against a particular strain is determined by the EC_{50} value, with a lower value indicating greater antimicrobial potency [9]. Based on the hazard assessment scores developed by Passino and Smith [10], the Im-SAILs were relatively harmless against all the six microorganisms tested in this study. Table 2 shows the results of antimicrobial susceptibility of the three Im-SAILs on various microorganisms, which was compared to the results of the broth microdilution assay and mutually correlated. According to the diameter of zone inhibition measurement developed by Jaganathan et. al. [8], all the three Im-SAILs weakly inhibited (+) the growth of *P. aeruginosa*, indicating their low potency against this microbe. 1-octyl-3-methylimidazolium tetrafluoroborate showed weak inhibition (+) against other Gram-positive bacteria such as *B. cereus* and *S. aureus*, while exhibiting good inhibition against fungi strains such as *A. fumigatus* and *C. albicans*. On the other hand, 1-decyl-3-methylimidazolium tetrafluoroborate and 1-dodecyl-3-methylimidazolium tetrafluoroborate demonstrated good inhibition against all Gram positive and negative bacteria and fungi, except for *Pseudomonas aeruginosa*. The toxicity of Im-SAILs towards microorganisms is influenced by the length of alkyl chain at the N-3 position of imidazolium cation [11]. This phenomenon has been observed in previous studies by Montalban et. al. [12], Kubo et. al. [13] and Kusumahastuti et. al. [14], which reported an increase in antimicrobial potency as the length of alkyl chain elongates. In our experiment, a similar trend was observed, with the toxicity of Im-SAILs increasing in the following order: 1-dodecyl-3-methylimidazolium tetrafluoroborate > 1-decyl-3-methylimidazolium tetrafluoroborate > 1-octyl-3-methylimidazolium tetrafluoroborate against microorganisms such as *A. fumigatus*, *B. cereus* and *P. aeruginosa*. Additionally, a similar trend was observed in the case of *C. albicans* and *S. aureus*, where 1-decyl-3-methylimidazolium tetrafluoroborate exhibited the highest toxicity, followed by 1-dodecyl-3-methylimidazolium tetrafluoroborate and finally, 1-octyl-3-methylimidazolium tetrafluoroborate. This observation could be attributed to a threshold effect caused by the increase in alkyl chain length, leading to the levelling off of toxicity after 1-decyl-3-methylimidazolium tetrafluoroborate. These findings align with previous studies by Kusumahastuti et. al. [14] and Kapitanov et. al. [15], which suggests that lengthening of the alkyl chain length of imidazolium cation resulted in the limited solubility of the compound and a lower fraction availability to disrupt the biological membrane of the microorganisms. Additionally, long-chain Im-SAILs mimic the plasma membrane of microorganisms, thereby reducing the potential impact of Im-SAILs on the lipid bilayer. Im-SAILs with longer alkyl chains, such as dodecyl chain, tend to self-aggregate and form micelles, which reduces the concentration of ILs at the action site. An unanticipated result was observed in the case of *E. coli*, where 1-octyl-3-methylimidazolium tetrafluoroborate exhibited the highest potency, followed by 1-dodecyl-3-methylimidazolium tetrafluoroborate and finally, 1-decyl-3-methylimidazolium tetrafluoroborate.

Table 1: EC_{50} values of Im-SAILS against Gram positive and negative bacteria, yeast, and fungi

Microorganism	Ionic Liquids (ppm) 1-octyl-3-methylimidazolium tetrafluoroborate	1-decyl-3-methylimidazolium tetrafluoroborate	1-dodecyl-3-methylimidazolium tetrafluoroborate
Aspergillus fumigatus (Fungi)	17489 (Lower limit: 14511; Upper limit: 21092)	16647 (Lower limit: 14452; Upper limit: 19181)	15878 (Lower limit: 12469; Upper limit: 20225)
Bacillus cereus (Gram positive bacteria)	27469 (Lower limit: 21406; Upper limit: 35375)	19906 (Lower limit: 16675; Upper limit: 23786)	17377 (Lower limit: 13752; Upper limit: 21963)
Candida albicans (Yeast)	18213 (Lower limit: 15256; Upper limit: 21756)	12809 (Lower limit: 10971; Upper limit: 14959)	15281 (Lower limit: 13381; Upper limit: 17456)
Escherichia coli (Gram negative bacteria)	18399 (Lower limit: 14543; Upper limit: 23290)	21994 (Lower limit: 18091; Upper limit: 26788)	19082 (Lower limit: 16216; Upper limit: 22467)
Pseudomonas aeruginosa (Gram negative bacteria)	20150 (Lower limit: 16145; Upper limit: 25193)	17787 (Lower limit: 14478; Upper limit: 21871)	14112 (Lower limit: 11616; Upper limit: 17148)
Staphylococcus aureus (Gram positive bacteria)	40794 (Lower limit: 30952; Upper limit: 54155)	6818 (Lower limit: 5209; Upper limit: 8936)	17820 (Lower limit: 14372; Upper limit: 22134)

Table 2: Antimicrobial disk diffusion of Im-SAILs against Gram positive and negative bacteria, yeast and fungi

Microorganism	Ionic Liquids (mm) 1-octyl-3-methylimidazolium tetrafluoroborate	1-decyl-3-methylimidazolium tetrafluoroborate	1-dodecyl-3-methylimidazolium tetrafluoroborate
Aspergillus fumigatus (Fungi)	11.67 (++)	14.33 (++)	16 (++)
Bacillus cereus (Gram positive bacteria)	9.67 (+)	12.33 (++)	14.33 (++)
Candida albicans (Yeast)	13.33 (++)	20.67 (++)	19.67 (++)
Escherichia coli (Gram negative bacteria)	14 (++)	13.67 (++)	13.67 (++)
Pseudomonas aeruginosa (Gram negative bacteria)	9 (+)	9.67 (+)	10.67 (+)
Staphylococcus aureus (Gram positive bacteria)	10.67 (+)	13.67 (++)	12.33 (++)

This discrepancy could potentially be attributed to the cut-off effect of the octyl chain of 1-octyl-3-methylimidazolium cation. The mode of action of Im-SAILs against bacterial cells involves the disruption of plasma membrane, leading to the progressive leakage of cytoplasmic materials to the external environment [16]. The mechanism of antifungal activity by Im-SAILs has been extensively studied in previous literature by McDonnell and Russell [17] and is similar to that of bacterial toxicity. Im-SAILs can penetrate the cellular membrane of fungi, disrupting the internal structure of the membrane, causing lysis, and inhibiting the formation of spores. Another hypothesis suggested the formation of micelle aggregates at the cell surface during the binding process between Im-SAILs and fungal cells, which has been further supported by the study conducted by Ahlstrom et. al. [18] on *C. albicans*.

4. Conclusions

In general, the results of the study revealed that based on the hazard assessment scores developed by Passino and Smith [10], the Im-SAILs were relatively harmless against all the six microorganisms tested in this study. The results of antimicrobial susceptibility of the three Im-SAILs mutually correlated with the microdilution assay. It was observed that the toxicity of Im-SAILs towards microorganisms is influenced by the length of alkyl chain at the N-3 position of imidazolium cation.

Acknowledgements

This research was funded by the Royal Society Commonwealth Science Conference 2021 Follow-on Grant (CSC\R1\211020). The authors would also like to extend their gratitude to QUEST International University for the laboratory facilities for this research.

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