Imidazolium and pyridinium based ionic liquids with dioctyl succinamic acid anion for Anti-bacterial Applications

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MS THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by: Syeda Hadeesa Kazmi, Regn No. 00000321238 Titled: Imidazolium and pyridinium based ionic liquids with dioctyl succinamic acid anion for Anti-bacterial Applications be Accepted in partial fulfillment of the requirements for the award of **MS** degree.

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To my parents for their unconditional love, inspiration, and support.

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In the name of Allah most merciful and the most beneficent. I am highly grateful to Him for his unlimited blessings He has always showered on me. I am thankful to Allah that he gave me knowledge to understand and health to work hard and complete my research wholeheartedly.

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Abstract

Six ionic liquids were synthesized with cation consisting of imidazole and pyridine with chain length of eight carbons. Anions were bromide and dioctyl succinamic acid. Ionic liquids were characterized by Fourier transform infrared spectroscopy (FT-IR) to ensure functional groups while molecular structure was ensured by nuclear magnetic resonance (NMR). These were tested for their anti-bacterial application and showed considerable activity against *S. aureus* and *E. coli* through agar well diffusion method.

Contents

Chapter 1

1. Introduction

1.1. Problem statement:

Bacteria have become major health concern now a days. They are present everywhere around us e.g. homes, hospitals, in air and also in human bodies. Some bacteria are beneficial for us but most of them are harmful and cause diseases in our body and animals. Bacteria cause community acquired infections and nosocomial; hospital acquired infections. Various antibacterial medicines have been developed to treat such infections but many bacteria have developed antibiotic resistance against antibiotics. Due to this resistance bacterial infections have become major cause of mortality because bacteria have tendency to grow into colonies within short period of time when they get favourable conditions. Other than antibiotics nanoparticles coatings have been developed but they have issue of metal deposition into the environment.

Another major issue is heavy metal pollution in water, soil and also air. Heavy metals like Pb, Hg, Cu, Cr, Zn, As have contributed a lot in environmental pollution through bioaccumulation in food chain and have become health concern and also death in organisms. Many techniques like membranes have been developed to capture and remove these metals but this is still an issue to be solved.

Ionic liquids are chemicals with low viscosity and vapour pressure. These are toxic for microbes but not for humans. Also there is no metal transfer into the environment from ionic liquids. This advantage can be used as antibacterial agent with high efficiency by penetrating into cell walls of microbes or incorporation into their DNA.

Ionic liquids are also useful for metal removal by ion exchange; cation exchange mechanism which considerably reduce risk of diseases in organisms thus becoming useful approach to solve problem of pollution caused by heavy metals.

1.2. What is Bacteria?

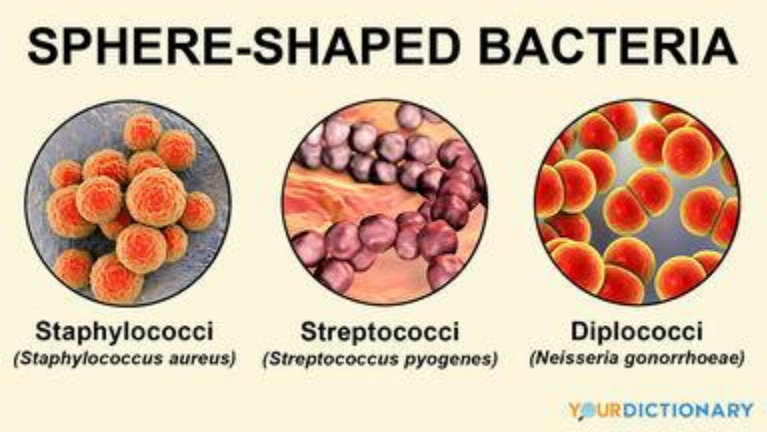
Bacteria are unicellular prokaryotic organisms that can live in diversity of environments, either independently in soil, water, air or in the host e.g. humans gut and plants etc. These are oldest organisms to be known as first fossils found were of bacterium. They range in few micrometre of length and exist as colonies of millions of bacteria together. Most of the earth is found to be made of bacteria because of their presence in every part of environment specially earth biomass.[1]

1.2.1. Types :

One way to classify bacteria is on basis of their **shapes** as follows:

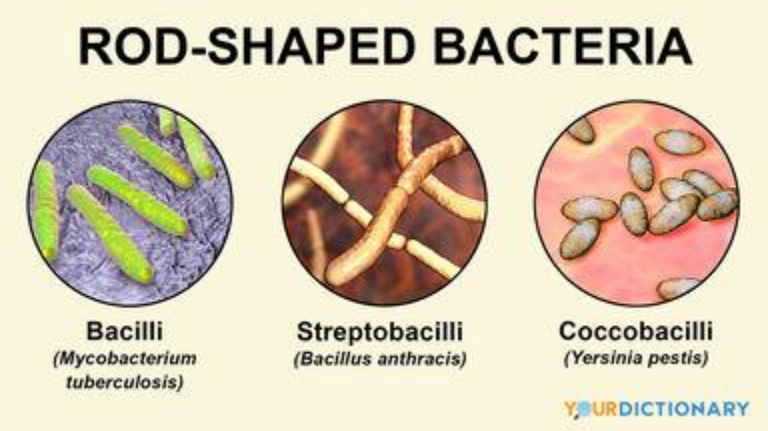
- **Spherical:**

  Spherical shaped bacteria are ball like called cocci. e.g. *streptococcus* that cause strep throat. Other examples include *Aerococcus urinae, Chlamydia trachomatis, Enterococcus faecalis* etc.



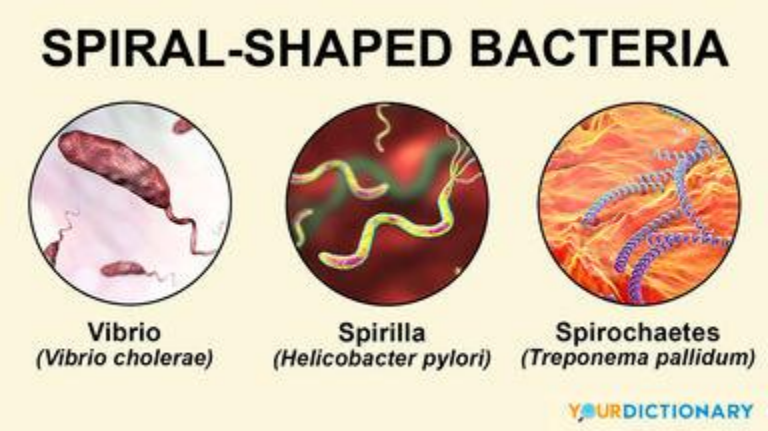
- **Rod-shaped:**

  Rod like bacteria is called *bacillus*. Curved shaped bacillus is called *vibrio* e.g. *Bacillus anthracis* or anthrax. Other examples are *E. coli, salmonella* etc.



- **Spiral shaped:**

  These are like spirals and if tightly packed called spirichetes. e.g. *Vibrio Cholerae, Helicobacter pylori, Treponema palladium, Camphylobacter jejuni etc.*



Classification on basis of cell wall:

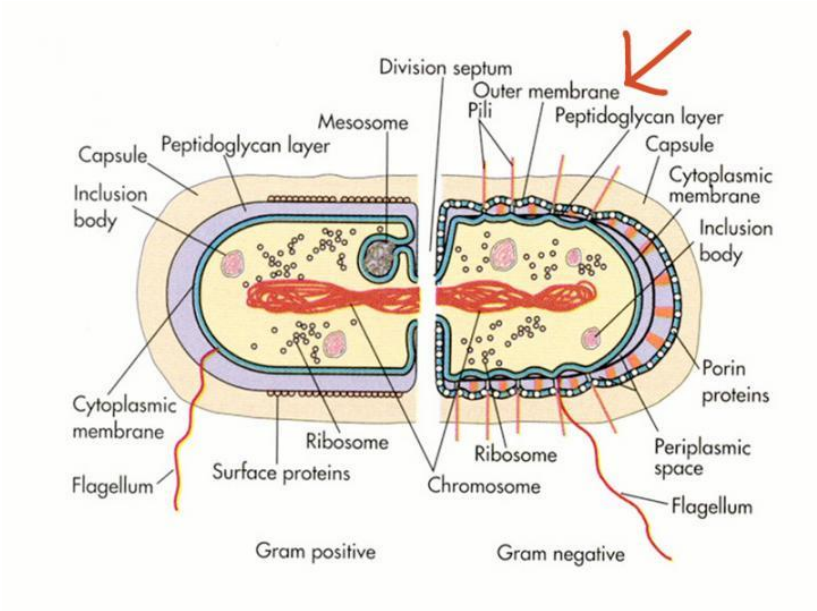
There are two types of bacteria on basis of cell wall types. Technique was developed by Hans Christian Gram in 1884.

- **Gram Positive:**

  Gram positive bacteria lack outer lipid layer and are stained violet when tested. For example, *S. aureus , S. epidermidis ,S.pneumonae etc*

- **Gram Negative:**

  These have thick outer lipid layer. They does not show violet stain after testing. For example, enterococci, salmonella species and pseudomonas species.



Classification based on gaseous requirement:

Aerobic bacteria:

These bacteria can grow in presence of oxygen. Can produce more energy. E.g. *Mycobacterium tuberculosis.* Aerobic bacterium cause environmental problems like corrosion, fouling and bad smells etc.

Anaerobic bacteria:

Anaerobes grow without oxygen and produce less energy. They are mostly found in gastrointestinal tracts of humans and cause diseases tetanus, gangrenes, botulism. e.g. *Bacteroids, E. coli, Clostridium* etc.

Facultative bacteria:

Bacteria that can exist with or without oxygen i.e. in aerobic or anaerobic conditions are said to be facultative e.g. *Escherichia coli, Pseudomonas aeruginosa.*

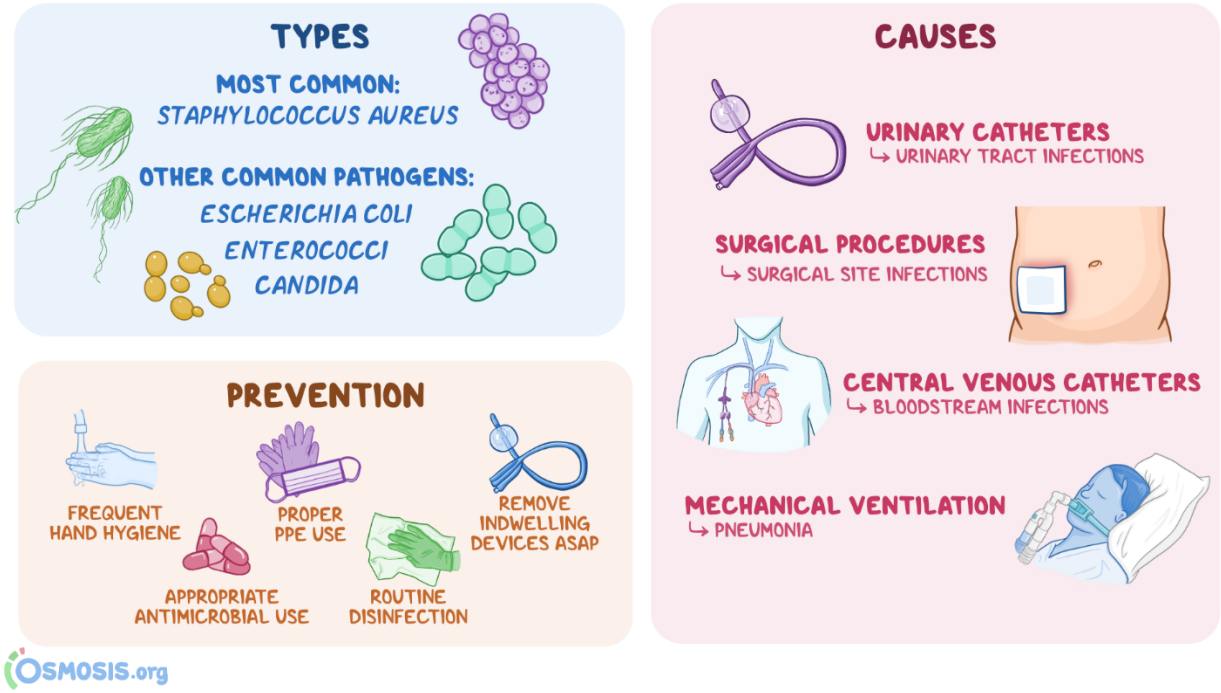
1.2.2. Bacteria and human diseases:

Bacteria are present everywhere around us. Some are beneficial but many of them are harmful for humans and cause serious health concerns. Different bacteria present in hospitals, homes and other places causing diseases are:

- *Escherichia coli*
- *Salmonella typhimurium*
- *Staphylococcus aureus*
- *Mycobacterium tuberculosis*
- *MRSA*
- *P. aeruginosa*
- *Colistridium difficile*
- *K. pneumoniae*
- *S. pneumoniae*
- *Haemophilus influenzae;*etc

Nosocomial infections:

Also called hospital-acquired infection; is acquired in health care unit.



Community acquired infections:

Such infections are caused outside the hospitals and are diagnosed within 48 hours of admission without any prior health care encounter.

- ***E. coli***

These are gram negative anaerobic bacterium. *E. coli* cause Hospital associated infections in the bloodstream and has gastrointestinal and urinary source. These sources should be targeted separately to reduce infections.[2] Infections caused by *E. coli* include diarrheal infections , as well as neonatal meningitis, haemolytic uremic syndrome, septicemia, and urinary tract infections (UTIs). These infections spread by eating bad food, contaminated drink or contacting infected person. E coli can proliferate within 10 hours to 6 days.[3]

- ***Staphylococcus aureus***

These are gram positive aerobic bacterium and due to high pathogenicity can cause pneumonia, skin infections, osteomyelitis and endocarditi, gastroenteritistoxic shock syndrome, scalded skin syndrome and acute suppurative inflammation (phlegmon), endocarditis, metastatic infection, sepsis syndrome [4]. These can cause both hospital and community acquired infections.

These bacteria have also developed resistance against antibiotics hence difficult to treat.

- ***Mycobacterium tuberculosis:***

M. tuberculosis either gram positive or gram negative causes pulmonary TB and extra pulmonary TB like pleural TB, lymphadenitis TB, ocular TB, gastrointestinal TB and skeletal TB.

Airborne pathogen caused "Great White Plague'' epidemic in England and western Europe and caused many deaths.[5]

- ***Pseudomonas aeruginosa:***

It is gram negative bacteria with thick peptidoglycan layer and cause nosocomial infections in patients who are admitted to the hospitals for long time; are more prone to its pathogenicity. It can cause infections like pneumonia, urinary tract infections malignant external otitis, endophthalmitis, endocarditis, meningitis. Antipseudomonal aminoglycosides and penicillins, Ticarcillin and carbenicillin for neutropanic patients are prescribed for such infections and are effective to much extent. [6]

For drug resistant *P. aeruginosa* "Colistin therapy'' was used but has adverse effect on kidneys of the patients causing renal failure in 58% of persons not having normal creatinine levels. [7]

- ***Klebsiella pneumonae:***

This gram negative bacteria cause hospital acquired infections that can be epidemic or endemic. Infections include are pneumonia, meningitis, bloodstream infections, urinary tract infections and surgical site infections. Amino-glycoside resistant strain can cause deaths especially in children. Transmission occurs from one person to another.

Monotherapy for three days is sufficient to treat *K. pneumonia.* Antibiotics cefroperazone. Sulbactam, piperacillin, tazobactam and imipenem are prescribed for its infections.

Monotherapy caused high mortality rates so combines therapy in which two or more drugd were associated and introduced in vitro. [8]

CAZ-AVI (Ceftazidime-Avibactam) Salvage Therapy is also effective against *K. pneumonia*.[9]

- ***Streptococcus pneumonia:***

S. pneumonia are gram positive aerobic diplococcic. These are numbered one to cause acute pneumonia in patients and severe cases may cause death other diseases caused are meningitis, pneumococcal empyema, endocarditis, pericarditis Septic arthritis, osteomyelitis, peritonitis. Brain abscess, pomposities, and peritonitis, are rare.[10]

- ***Haemophilus influenza:***

Gram negative facultative anaerobic bacillus *H. influenza* can cause mild to severe infections e.g. respiratory tract infections including otitis media, pneumonia, epiglottitis, eye infections, bloodstream infection and, meningitis. It can also cause infectious arthritis and cellulitis (skin infection).

Minimum inhibitory concentration (MIC):

Minimum concentration of the chemical substance enable to inhibit growth of bacteria visually. It is measured in µg/ml or mg/L.

The microorganism, the antibiotic itself and the affected human being (in vivo only) are the factors affecting MIC value.

1.2.3. Minimum bactericidal concentration:

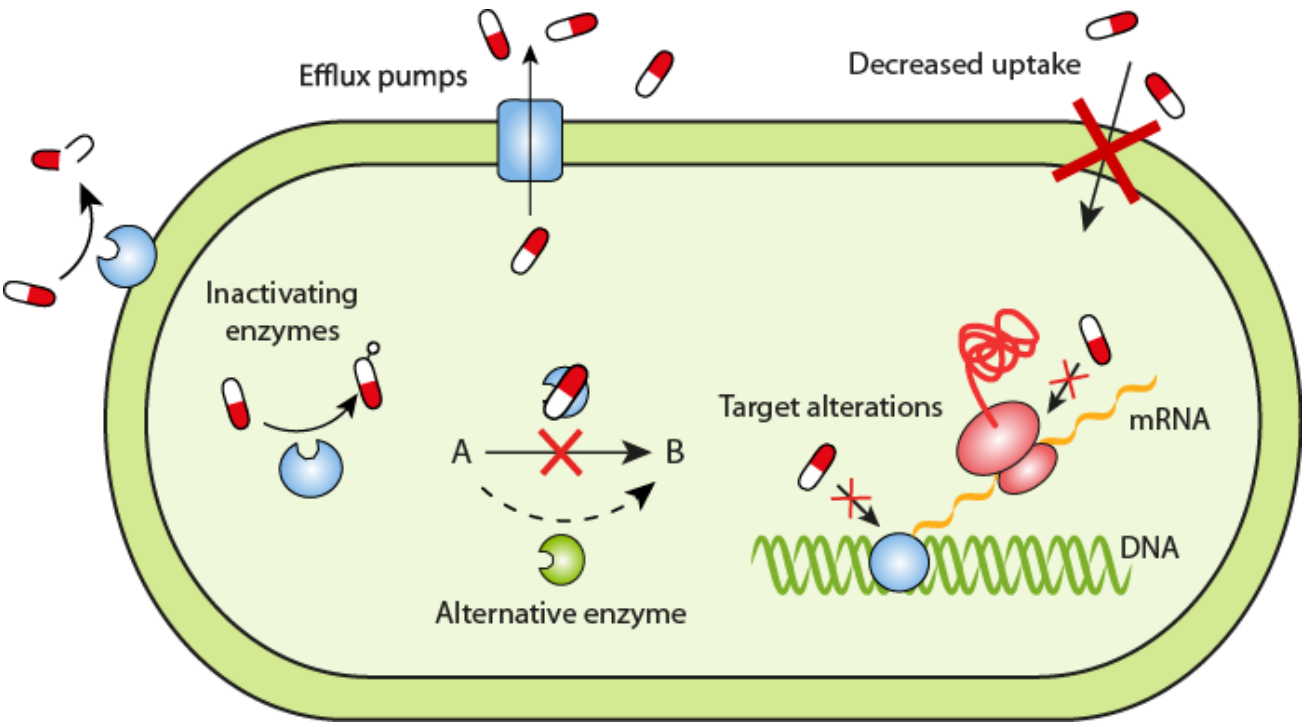
Minimum concentration of agent that can kill bacteria.

Closer the MIC is to the MBC, the more bactericidal the compound. For the compound to be an effective bactericidal, it is necessary that the difference between the values of MIC and MCB is less.

Minimum inhibitory concentration values are important in determining antibacterial efficiency of antibiotics.

Bacteria	**MIC(µg/ml)**
E.coli	MIC_{50}=0.25 and 0.5
	MIC_{90}=0.5 and 1
Staphylococcus aureus	≤2 for vancomycin stain
Mycobacterium tuberculosis	0.03–0.12 for isoniazid
Pseudomonas aerugonosae	≤16 for piperacillin[11]
Klebsiella pneumonae	7.0[12]
Streptococcus pneumonia	≤0.19 − 0.1.5[13]

1.2.4. Antibiotic resistance of bacteria:



Several bacteria have gained resistance against most of the antibiotics that have made bacterial infections much more adverse than before. Due to these antibiotic resistance many therapeutic treatments have failed. This have caused difficulty to treat infections and deteriorated human health.[14]

1.3. Heavy metals and pollution:

Heavy metal pollution has emerged due to anthropogenic activity which is the prime cause of pollution, primarily due to mining the metal, smelting, foundries, and other industries that are metal-based, leaching of metals from different sources such as landfills, waste dumps, excretion, livestock and chicken manure etc.

1.3.1. Water pollution by heavy metals:

Heavy metals bio accumulate in tissues of organisms with passage of time after their uptake due to slow metabolism for their excretion. Common sources of water pollution are water supply by industrial and consumer waste , acid rain that leaches the soil and increase heavy metal concentration in the water.[15]

Most frequently present heavy metals in water are Chromium (**Cr**), lead (**Pb**), arsenic (**As**),

Mercury (**Hg**), nickel (**Ni**), iron (**Fe**), zinc (**Zn**) and vanadium (**Va**).

1.3.2. Soil pollution by heavy metals:

Hg and **Cd** are major accumulating metals beside Cr, Zn, Ni, Pd etc. caused by processes of mining, smelting. [16]

1.3.3. Air pollution by heavy metals:

Heavy metals cause air pollution by emission from coal industry, smoke of vehicles. In Jharkand, India Cu, Fe, Mn, Zn, Pb, Cd, Cr and Ni were found to be potential pollution hazards.[17]

1.4. Diseases caused by heavy metals:

Cd can cause renal failure, lung diseases and defected bones.

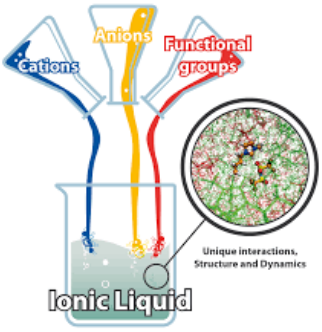
Pb is most important to cause illness like inhibits haemoglobin formation; kidney failure, reproductive systems and dysfunction of joints, acute and chronic damage to the central nervous system and cardiovascular system.

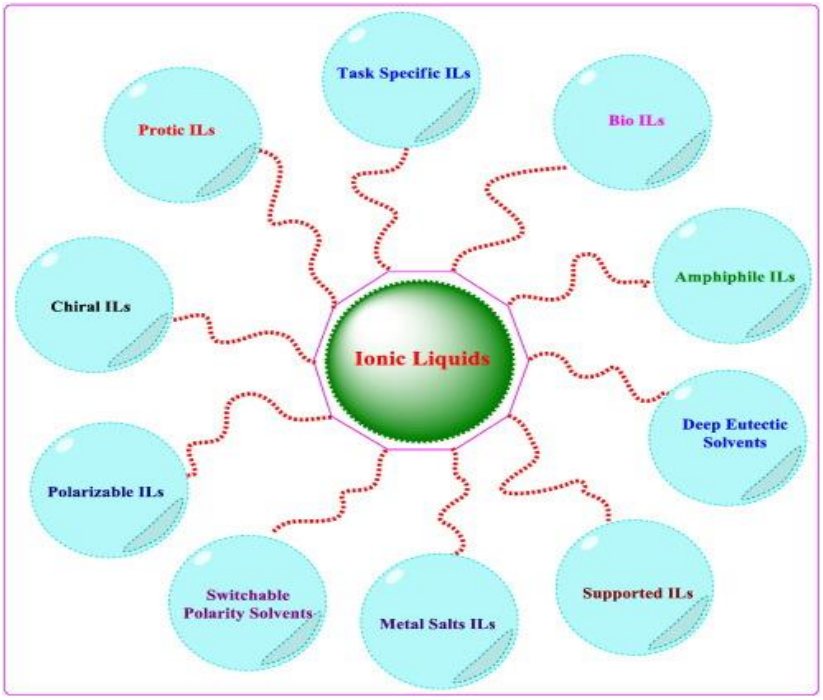
Zn can cause impaired reproduction and growth, diarrhoea, vomiting, bloody urine, liver failure, anaemia and kidney failure.

Hg causes congenital malformation, spontaneous abortion, and GI disorders. Organic mercury compounds are cause of erethesis.

1.5. Ionic Liquids:

Chemical substances that are liquid under temperature of 100 degree celcius; consisting of organic part as cation and anion may be organic or inorganic. Ionic liquids have vast applications due to their different types as shown in figure:





These are also called **ionic melts, liquid electrolytes**, **ionic fluids**, **liquid salts**, **fused salts** or **ionic glasses.** These possess vast applications like:

- Electrolytes
- Electric batteries
- Antimicrobial or antibacterial agents
- CO_2 capture
- Pharmaceuticals
- Nuclear fuel reprocessing
- Waste recycling
- Bipolar processing
- Heavy metal uptake ; etc

1.5.1. Ionic liquids properties:

Ionic liquids have many useful properties most important of them is toxicity that acts an antibacterial activity. Other properties include:

- Melting point:

  As defined ionic liquids have melting point less than 100ºC.

- Viscosity :

  Ionic liquids have high viscosity than conventional organic solvents due to strong van der waals forces, hydrogen bonding and alkyl chain lengths.

- Thermal stability:

  These have high thermal stability; can endure high temperatures. The thermal stability of ionic liquids is <225 °C.

- Density:

  These liquids have high density than water and other organic solvents.

- Vapour pressure:

  Ionic liquids have negligible vapour pressure.

- Antibacterial or antimicrobial nature:

Bacteria have become resistant to antibiotics through formation of biofilms. Toxicity of ionic liquids is important and can prevent bacterial growth when paints with ionic liquids is done on surfaces.

Chapter 2

2. Literature review:

A lot of work has been done and published on antibacterial and heavy metal removal through different techniques to draw attention towards these problems that have created adverse effects on human health and daily life.

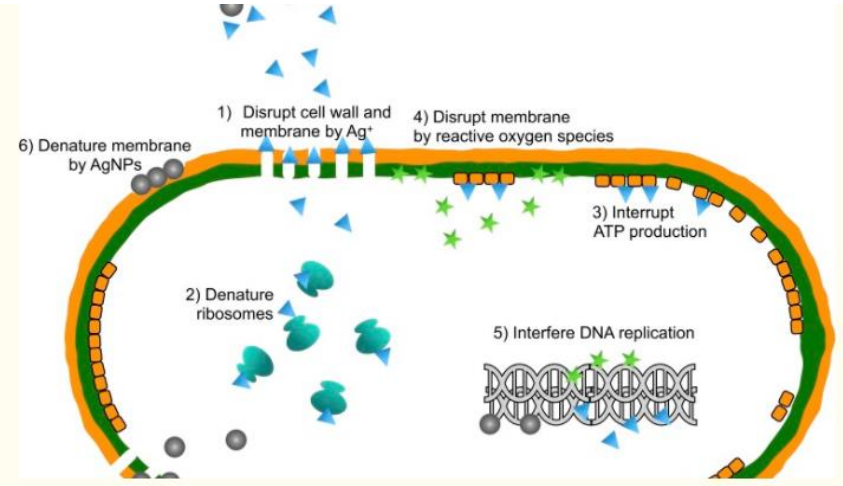
Various approaches have been made to make antibacterial agents i.e. nanoparticles of Cu, Zn, Fe, Mg, Ag etc. are used.

2.1. Nanoparticles based antibacterial agents:

Bondarenko, O. *et al* studied toxic effects of CuO, ZnO and nanosilver NPs for their efficiency to stop bacterial growth in mamalian cells. MIC values for bacteria were 500, 200 and 7.1 mg/L for ZnO, CuO and Ag NPs respectively. L(E)C_{50} value for Ag , CuO and ZnO NPs were calculated to be <10mg/L, >100mg/L and 10-100mg/L respectively. Thus Ag nanoparticles were most batericidal. Drawback to use nanoparticles to targetted species was leaching of the metals to water thus causing toxicity to aquatic life. [18]

Yin, I. X. *et al.* have reported mechanism of Ag NPs as bactericides and observed NPs to be more toxic against gram negative bacteria as compared for gram positive one due to thin outer layer. NPs release Ag ions on bacteria and kill them by breaking their cell walls. Ag particles below

10nm are more effective in antibacterial application.Concern are free Ag ions that enter environment and cause adverse effects due to their toxicity. Mechanism devised is shown in following figure: [19]



Lee *et al.* studied a new mechanism for the of bacterial called "apoptosis-like response'' against *E.coli* that was more effective than using antibiotics against which resistance have been developed by bacteria. Average size of 3nm for Ag was measured by TEM. Bacterial death was observed after 6h incubation with NPs. Method was useful for both gram negative and gram positive bacteria. [20]

T. Bhatti *et al.* made Cu^{o} NPs through inert gas condensation method against *E. coli*. Size of 12nm was characterized by XRD and TEM method. SEM revealed interaction of Cu and gram negative *E. coli* resulted in formation of pits in the bacterial call wall through reduction reaction. NPs with large surface to volume ratio are more efficient antibacterials. [21]

Y. Liu *et al.* studied ZnO NPs for their antibacterial activity against *E. coli* strain O157:H7. Greater

the NPs concentration, greater antimicrobial efficiency through cell wall breakage of bacteria. [22]

T. Webster studied effect of Se NPs against *S. aureus* gram positive bacteria after incubation at 7.8, 15.5, and 31 µg/mL concentration for 3, 4 and 5 hours. These NPs were proved to be efficient to treat and inhibit infectious diseases caused by this antibiotic resistant bacteria. [23]

S. Thakur *et al.* prepared Ag doped ZnO NPs for antibacterial activity against *S. aureus* and *B. subtilis* and tested by XRD. Greater Ag concentration, smaller is the ZnO grain size, more antibacterial effect against *S. aureus*. [24]

M. Joice *et al.* synthesized ZnO NPs against antibiotic resistant *K. pneumonia* using Hep2 cell line by precipitation method and characterization done by SEM and XRD methods. MIC values were calculated to be 40µg/ml. it was observed that 0.75mM Nps of ZnO inhibited *K. pnmoniaeu* after 4h incubation. These NPs were effective at dosage of 0.50 and 0.75 mM. [25]

A. Anwar *et al.* observed conjugated chlorhexidine (Au–CHX) with *gold* nanoparticle against *K. pneumoniae* isolates; characterized by UV-VIS spectroscopy, ESI-MS, AFM and FT-IR techniques. These NPs completely inhibited biofilm synthesis of bacteria at concentration of 25 and 100µM due to which they develop antibacterial resistance At 75 and 100µM concentration NPs destroyed already existing biofilms; thus preventing HAI by this bacteria.[26]

M. Kopciuch *et al*. observed that biofilm synthesis was stopped in *P. aeruginosa* by FeO NPs coated with alginate and magnetite–tobramycin conjugates coated with alginate. [27]

M. Goswami *et al.* prepared ZnO NPs for antimicrobial and anti-biofilm applications against *S. pneumoniae*. Antibacterial activity was exhibited at MIC 40µg/ml and anti-biofilm ability at sub MIC value of 3, 6 and 12µg/ml with maximum efficacy at 12µg/ml of ZnO concentration. [28]

2.2. Ionic Liquids as antibacterial agents:

Despite high activity of NPs as antibacterials , they have a drawback of leaching of metals into the environment thus incorporated into the food chain causing bio accumulation and producing toxicity and adverse issues like kidney failure, nervous system damage, liver diseases etc. More efficient and less harmful chemicals were required to replace the practice of using NPs that should be helpful in addressing the nosocomial and community acquired infections. For this purpose Ionic Liquids have served important role because they are not only more antibacterial but also are less toxic to humans. Many research work has been done to support this hypothesis.

K. Zohdey *et al.* made two ionic liquids namely 1-ethyl-3-methyleimidazolinium chloride ([EMIm]Cl) and 1-(2-hydroxyethyl)-3-methylimidazolinium chloride ([OH-mim]Cl) for antibacterial and anti-corrosive activities. Out of these two ionic liquids [OH-mim]Cl was more biocidal due to OH group present in the alkyl side chain. Bacterial growth was observed to stop completely after 6h incubation of 12ppm dose of [OH-mim]Cl ; most effective at 100ppm concentration of ionic liquid in 1h by destroying cell membrane of bacteria. High concentration of ([EMIm]Cl) is needed for biocidal action. [29]

K. Islam studied antibacterial multi-cationic bisimidazolium and benzimidazolium ionic liquids with di, tri and tetra alkyl chains against both gram negative and gram positive bacteria characterized with IR and NMR spectroscopies. Dicationic ionic liquids showed considerable results. [30]

Q. Xu et al. reported pyrolidinium based small IL and poly IL with four, six, eight and ten carbon chain length for antibacterial application against *E. coli* and *S aureus*. Increasing chain length decreased MIC value for simple ILs thus increasing their antibacterial activity. Case is opposite

for poly ILs whose activity decrease with increase in side alkyl chains. [31]

Zhou et al. prepared imidazolium salts (IMS) polymers and hydrogels for antimicrobial activity against both gram positive (*MRSA* and *S. aureus*) and gram negative (*PA01* and *E.coli*) bacteria characterized by SEM and FTIR and having MIC_{90} value less than 2µg/ml. hydrogels killed 96.1% of *PA01* bacteria and were nontoxic to humans. [32]

M. Vrablova et al. formed four ionic liquid;, octyltriethylammonium bis(trifluoromethyl-sulfonyl)imide, butyltriethylammonium NTf_2, dodecyltriethylammonium, NTf_2, butyl-3-methylimidazolium NTf_2, and their bromide precursors for biocidal and antibacterial activity. Bromide IL showed maximum antibacterial activity. These ILs were applied in paints, coatings, antibacterial cleaners and disinfectants. [33]

Sofy et al. prepared ionic liquids-based Schiff bases (BBITSB and SITSB) for antifouling and antibacterial applications against biofilm forming bacteria gram *A.hydrophilia* and *E. coli*. They used these ionic liquids in paints [34]

F. D'Andrea et al. studied fifteen ILs out of which they formed four ILs; 1-dodecyl-1-methylpyrrolidinium bromide, 1-methyl-3-dodecylimidazolium Br^- and 1-dodecyl-1-methylpiperidinium for against gram positive and gram negative bacterium *Staphylococus aureus* and *Pneumonia aeruginosa*. ILs exhibited low MIC values and killed both bacteria at MBC (minimum bactericidal concentration) value by inhibition of biofilm formation that cause antibiotic resistance. [35]

S. Stolte et al. evaluated the antimicrobial activity of ILs through quantitative structure activity relationship models and linear free energy relationship descriptors against *S. aureus* and *E. coli* and determined MIC values for them. They found IL cations to be more biocidal for bacteria than

anions. [36]

K. Docherty and C. Kulpa evaluated activity of 4, 6 and 8 carbon chain imidazolium and pyridinium bromide ILs against, *Staphylococcus aureus, Pseudomonas fluorescens, Bacillus subtilis,* and *Saccharomyces cerevisiae, Escherichia coli* through Microtox method. More antibacterial the IL was exhibited with increasing chain length. [37]

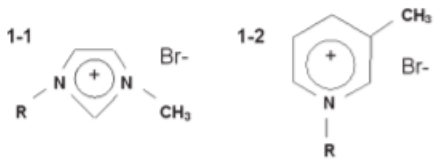


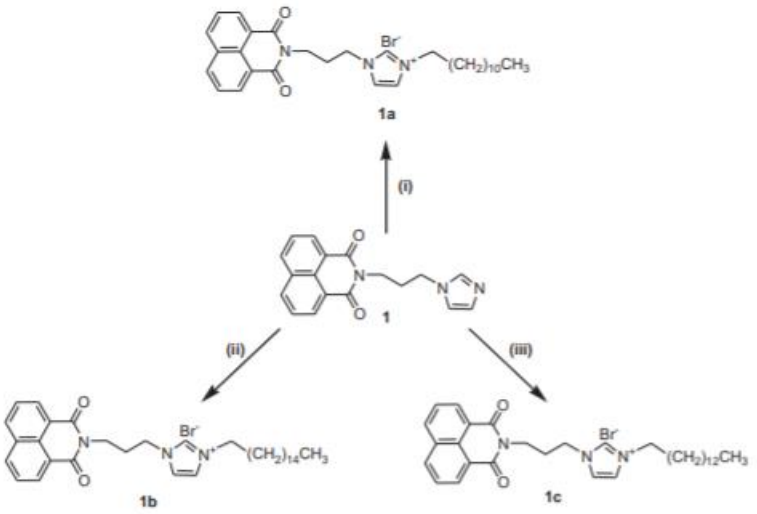
Fig. 1 Chemical structure of 1-alkyl-3-methyl imidazolium (1-1) and 1-alkyl-3-methyl pyridinium (1-2) bromide.

B.Mokhtarani et al. studied effect of pyridinium and imidazolium ILs, [HMIM][PF6], [HMIM][I], [HMIM][Cl],], [HMIM][NTF2], [HMIM][BF4 [HMIM][PTS],], [HMIM][NO3], [HPY][NO3], [BPY][NO3], [BMIM][NO3] ,[OMIM][NO3] and [HDMIM][BF4] against, *Salmonella Staphylococcus, Klebsiella pneumoniae, aureus typhimurium, , Pseudomonas aeruginosa Escherichia coli,* , and *Bacillus subtilis, Bacillus tequilensis*. Out of these 1-Hexyl-3-methylimidazolium [HMIM][NO3] and [HPY][NO3] were most antibacterial due to long alkyl chains. [38]

A. Tunçel et al. studied lipophillic imidazolium ILs salts of bromide; NIM-Br with different alkyl lengths against gram negative and positive pathogenic bacteria by determining their MIC values as follows: [39]

Minimum inhibitory concentration for each of the compounds and Gentamicin.

Organism	ITFSI (μM)	NIM-Br (1a) (μg/ml)	NIM-Br (1b) (μg/ml)	NIM-Br(1c) (μg/ml)	GEN (μg/ml)
Staphylococcus aureus **ATCC 29213**	1.875 ± 0	8.33 ± 2.89	1.04 ± 0.36	4.17 ± 1.44	0.5 ± 0
Escherichia coli **ATCC 25922**	1.875 ± 0	260 ± 90.07	2.08 ± 0.72	8.33 ± 2.89	0.5 ± 0
Pseudomonas aeruginosa **ATCC 27853**	3.75 ± 0	312 ± 0	80 ± 0	53.3 ± 23,09	1 ± 0
Enterococcus faecalis **ATCC 29212**	3.75 ± 0	8.33 ± 2.89	1.25 ± 0	8.33 ± 2.89	8 ± 0



Scheme 1. Synthesis of the imidazolium bromide salts, NIM-Br (1a, 1b, 1c). (i, ii, iii) $CHCl_3$, inert atmosphere, reflux, overnight, 1-bromododecane (for 1a), 1-bromohexadecane (for 1b), 1-brumotetradecane (for 1c).

J. Guo et al. formed IL monomers, PIL and PIL membranes with mono or bis IM cations for activity as against bacterias; *Escherichia coli* and *Staphylococcus aureus* devising their MIC values of various ILs.

Table 1. Antimicrobial Activities of Imidazolium-Type IL Monomers and PILs Measured as MIC

samples	MIC (μmol mL^{-1})	
	S. aureus	*E. coli*
IL-C2	472.906	945.812
IL-C4	54.545	54.545
IL-C8	2.983	1.192
IL-C12	0.038	0.061
IL-C6-Im-C2	110.599	55.300
IL-C6-Im-C4	27.273	22.945
IL-C6-Im-C8	0.081	0.081
IL-C6-Im-C12	0.018	0.018
PIL-C2	110.345	110.345
PIL-C4	2.961	5.922
PIL-C8	1.491	1.192
PIL-C12	0.061	0.122
PIL-C6-Im-C2	33.180	33.180
PIL-C6-Im-C4	0.918	1.853
PIL-C6-Im-C8	0.081	0.041
PIL-C6-Im-C12	0.009	0.018

Larger alkyl chain and more dense (bis- imidazolium) ILs have lower MIC values and more antibacterial.[40]

H. Seifert et al. synthesized twelve BIOnic liquids having imidazolium cations with C4, C8 and C16 chain lengths with bromide and antibiotic counter anions and gave MIC values against bacterial strains and found [C16mim]sulfadiazin and [C16mim]fosfomycin against *S. aureus* or [C16mim]sulfamethoxazol against *E. coli*) to me more effective. [41]

Table 3. Exemplary efficiency MIC (average) in μg mL^{-1} of [C_{16}mim]fosfomycin in comparison to its key components. MIC (average) is the sum of the five MICs against our five bacteria reference strains MIC (total) devided by 5.

Compound	MIC (total)	MIC (average)
[C_{16}mim]Br	161.25	32.25
Na_2 fosfomycin	58	11.6
[C_{16}mim]fosfomycin	41.25	**8.25**

F. Comelles et al. studied 1-alkylpyridinium bromides and [$C_n$mim] derivatives having C8, C10,

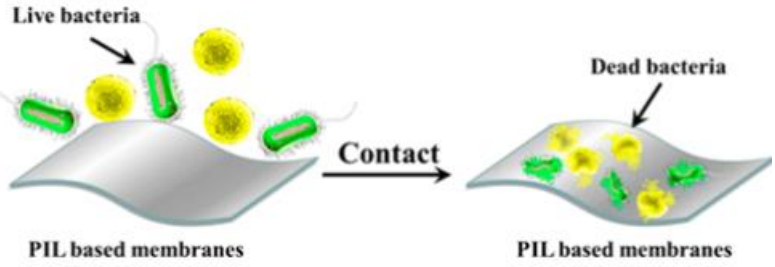
C12 and C14 carbon chains and effect of chain length for antimicrobial application and determined their MIC values of gram positive and negative bacteria. Long chain length ILs were more antibacterial. [42]

Microorganisms strain	MIC* (μM)			
	C_8mimBr	C_{10}mimBr	C_{12}mimBr	C_{14}mimBr
Gram-positive cocci				
Micrococcus luteus	R	R	R	178
Staphylococcus epidermidis	930	844	193	6
Staphylococcus aureus	R	106	97	45
Gram-negative rods				
Escherichia coli	R	R	386	356
Klebsiella pneumoniae	R	R	773	356
Pseudomonas aeruginosa	R	R	R	356
Fungi				
Candida albicans	R	R	R	178
Bacillus				
Bacillus subtilis	R	422	48	6

MIC values for 1-alkylpyridinium bromides (C_nPyrBr).

Microorganisms strain	MIC (μM)			
	C_8PyrBr	C_{10}PyrBr	C_{12}PyrBr	C_{14}PyrBr
Gram-positive cocci				
Micrococcus luteus	R	R	R	90
Staphylococcus epidermidis	940	428	49	6
Staphylococcus aureus	R	428	195	22
Gram-negative rods				
Escherichia coli	R	428	97	45
Klebsiella pneumoniae	R	R	780	359
Pseudomonas aeruginosa	R	R	780	359
Fungi				
Candida albicans	R	R	R	359
Bacillus				
Bacillus subtilis	R	428	24	6

Zheng et al. synthesized PIL membranes for bactericidal action and used amino acid anions of proline or tryptophan for both gram positive *S. aureus* and gram negative *E. coli* with no toxicity to humans. PIL-Pro$^-$ was less antibacterial than PIL-Trp$^-$. [43]



J. Bhinder et al. made IL-1d@MWCNT (IL functionalized multiwall CNTs) for antibacterial application against *Escherichia coli* and gram positive (*MRSA* and *S. aureus*) bacterial strains and characterized them via, X ray diffraction, SEM and FTIR. These were used in coatings that were self-sterilizing and prevent nosocomial infections in hospitals. [44]

Q. Ge et al. studied N-alkyl imidazolium PIL nanoparticles for *E. coli* and *S. aureus* with varying

chains and activity varying with chains as C8<C10<C10<C12. These ionic liquid based NPs act as antimicrobials by destroying bacterial cell wall. Also more hydrophobic NP was more antibacterial.[45]

H. Mao et al. formed PIL-Br poly (3-butyl-1-vinylimidazolium bromide) with anion, ($TPESO_3^-$) based on imidazole through anion exchange method and characterized it through ^{1}H-NMR and SEM.

Antibacterial activity was controlled through $Br^-/TPESO_3^-$ concentration. Smaller the ratio, more antibacterial are the ionic liquids. AIE (aggregation induced emission) type AIE-PIL are effective anti-biofilm agent. [46]

A.Abbaszadegan and coworkers synthesized AgNPs coated with Pyridinium and Imidazolium ILs with chain lengths (C12 & C18); ([C12mim]Cl), ([C18mim], 1-dodecyl pyridinium chloride ([C12Py]Cl) and ([C18Py]Cl compared to NaOCl and cyclohexidine (CHX) and found AgNPs coated with to be more antibacterial against bacteria *Enteroccocus faecalis*. [47]

The MIC_{90} of the experimented solutions

Solution	NaOCl	CHX	C_{12} Py	C_{18} Py	C_{12} Im	C_{18} Im
MIC $_{90}$ (M/L)	3.35×10^{-1}	4×10^{-3}	$8.1x10^{10}$	$8.5x10^{-9}$	$7.1x10^{-9}$	$8.1x10^{-9}$
Cell viability (%)	37.7849	3.26494	97.6678	99.1489	106.613	102.099

Z. Zheng and coworkers prepared PILs, 1-octyl-3-vinylimidazolium bromodichlorocuprate, 1-octyl-3-vinylimidazolium bromide, 1-octyl-3-vinylimidazolium bromodichlorozincate, 1-octyl-3-vinylimidazolium bromotrichloroferrate, and membranes for antibacterial activity and showed trend as IL-Cu > IL-Zn > IL-Fe > IL-Br (for *S. aureus* and *E. coli*) proved enhanced antimicrobial effect by metal anions.[48]

Table 1. Antibacterial Activities of Imidazolium-Type ILs Coordinated with $CuCl_2$, $FeCl_3$, and $ZnCl_2$ Measured as MIC

samples	MIC (μmol mL^{-1}) *S. aureus*	MIC (μmol mL^{-1}) *E. coli*
IL-Br	2.610 ± 0.003	1.321 ± 0.002
IL-Cu	0.056 ± 0.002	0.222 ± 0.001
IL-Zn	0.886 ± 0.003	0.886 ± 0.002
IL-Fe	1.254 ± 0.005	1.110 ± 0.003

M. Yu et al. synthesized diketopyrrolopyrrole-based (ILDs) having 1.95–4.2 nm molecular sizes against gram negative bacteria *E. coli* and *P. aeruginosa*. Size was important in determining antibacterial efficiency by penetrating and damaging thick wall of gram negative bacteria. LD-6 and LD-12 were most antibacterial while LD-8 due to smaller size can penetrate through cell wall and was less effective. [49]

Figure 6. Membrane damage mechanism of different ILDs against Gram-negative bacteria

2.3. Ionic Liquids for heavy metal purification:

D. Kogalnig et al. studied four ILs, trihexyl(tetradecyl)phosphonium-2-(methylthio)benzoate[PR4][MTBA], trihexyl(tetradecyl)phosphonium thiosalicylate [PR4][TS] tricaprylylmethylammonium-2-(methylthio)benzoate [A336][MTBA] and tricaprylylmethyl-ammonium thiosalicylate [A336][TS] to remove Cu, Cd, Pb, Cr, Ni & Zn and ≥90% efficiency for Ni, Cu and Zn from industrial waste water. [PR4][TS] was more efficient than [PR4][MTBA].

[50]

A. Stojanovic and B. Keppler reviewed that imidazolium based RTILs were effective for the removal of alkali metals, Zn, Co, Mn, Ni, through aqueous solutions via cation exchange mechanism. [51]

Germani et al. studied different [Cnmim] [PF6]; 1-alkyl-3-methylimidazolium hexafluoro-phosphate ILs as extracting agents for Hg through aqueous solution. C-4, 6 & 8 were used as extracting agents and [C8MIM] [PF6] showed 90% of Hg (ll) extraction in 4h and complete removal after 12h. [52]

M. Mahmoud and H. Al-Bishri extracted Pb metal through water samples with solid phase extraction via physical sorption using IL 1-methyl-3-octylimidazolium bis(trifluoromethyl-sulfonyl)imide [OMIM+Tf2N−] with 98-100% efficiency. They used multi-stage micro-column separation with nano silica [NSi-OH-OMIM+Tf2N−] or [NSi-NH2-OMIM+Tf2N−]. [53]

Urszula Domanska and Anna R, ekawek reported imidazole ionic liquids (ILs): 1-ethyl-3-ethylimidazolium, or 1-hexyl-3-ethylimidazolium or 1-butyl-3-ethylimidazolium bis{(trifluoro-methyl)sylfonyl} imide, or 1-butyl-3-ethylimidazolium hexafluorophosphate or 1-butyl-3-methylimidazolium hexafluorophosphate, 1-hexyl-3-ethylimidazolium hexafluoro-phosphate and dithizone chelator for the extraction of Ag^{+} and Pb^{2+} with solvent extraction technique and 93-97% efficiency for Ag ions via cation exchange mechanism. As C-atoms in alkyl chain increase, decreases extraction efficiency of metal ions. Extraction proceeded with passing of complexes of metals b/w aqueous and organic IL phases. [54]

Table 1 Solvent effect on the extraction of Ag^{+} at pH = 1.5, $T = 296$ K, sample 0.5 mL: chloroform (CH_3Cl) and ionic liquids {[EEIM][NTf_2], or [BEIM][NTf_2], or [HEIM][NTf_2], or [BEIM][PF_6], or [BMIM][PF_6], or [HEIM][PF_6]}

Solvent	$(C_i)_w$/ppm	$(C_f)_w$/ppm	C_o/ppm	$\%E$
$CHCl_3$	29.629	1.830	27.799	93.8
[EEIM][NTf_2]	29.630	0.211	29.419	99.3
[BEIM][NTf_2]	29.630	0.220	29.410	99.3
[HEIM][NTf_2]	29.629	0.338	29.291	98.9
[BEIM][PF_6]	29.629	0.403	29.226	98.6
[BMIM][PF_6]	29.629	0.325	29.304	98.9
[HEIM][PF_6]	29.629	0.695	28.934	97.6

S. Boudesocque et al. prepared novel TSIL by combining ester derivatives of betain and anions chlorosalycilate (ClSal), dicyanamide (Dca−), and saccharinate (sac). Extraction was done with both the cation exchange and ion pairing. Clsal was good for extracting Nickle, Copper, Lead, Cadmium and while dicyanamide was effective for Cu, Ni, Cd and Co. Saccharinate was efficient for Cd ions. These were characterized by UV-Vis spectroscopy. Also these ionic liquids can be used again and again through back-extraction.[55]

M. Priadarshini et al. used deprotonation metathesis method to prepare nine TSILs to remove heavy metals Fe, Ni, Cu, Pb and Zn through industrial waste water bodies. Ionic liquids prepared were, tetrapropylammonium salicylate, tetrapropylammonium benzoate, tetrabutylammonium benzoate tetrapropylammonium anthranilate,tetrabutylammonium salicylate, tricaprylmethylammonium benzoate, tetrabutylammonium anthranilate, tricaprylmethylammonium anthranilate and tricaprylmethylammonium salicylate . ILH was most efficient for Zn removal, ILF for Nickle, ILC~ILH for lead, ILA for iron and ILI for copper measured by Flame atomic absorption spectroscopy. This method was proved better than chemical precipitation, adsorption, reverse osmosis, electrolytic recovery and evaporator recovery process.

56

J. Motonaka et al. prepared N,N-diethyl-N-methyl-N-(2-methoxyethyl) ammonium bis(trifluoromethan-sulfonyl)imide with 8-HQ (8-hydroxyquinoline) chelator to extract zinc, manganese, cadmium, and copper heavy metals. [57]

	Concentration in sample solution[a] ($mmol\ l^{-1}$)	Extraction efficiency (%)	Recovered amount (μg)
Zn	9.2	99.98	301
Cd	10.1	99.68	567
Mn	9.6	97.98	258
Cu	9.4	100.0	298

A.P. de los R1´os and co-workers separated zinc, cadmium and ferric ions via ILs: [C_8 mim^+] [BF^{4-}] and methyltrioctylammonium chloride, [CmtA+][Cl-]. Former IL gave separation efficiency >90% and later was better to extract (>90%) zinc and cadmium ions from aqueous solutions.[58]

Koen Binnemans and co-workers separated trivalent ions and rare earth elements via betainium bis (NTf2) using phase transition extraction method. This functionalized IL was proved effective than conventional extractants.[59]

J. Yao et al. separated heavy metals from sewage sludge using methylimidazolium chloride ([mim]Cl, triethylammonium hydrogen sulfate ([TEA][HSO4]) and dimethylbutylammonium hydrogen sulfate ([DMBA][HSO4]). [mim]Cl separated nickel, cadmium, zinc and lead with >90% effectiveness while [DMBA][HSO4] extracted efficiently Cr (lV),Cr (lll) and arsenic ions.[60]

W. Reichert et al. synthesized urea and thio-urea derivatives of [1-alkyl-3-methyl-imidazolium] [PF6] (R = n-C_4 – C_8) TSILs for separating mercury and cadmium ions from aqueous solution. TSIL/[C4mim][PF6] gave better separation efficiency. Hg was better extracted with urea and thio urea derivative TSIL.[61]

G. Chauhan and A. De Klerk prepared ILs C_2MIM-DEP, DIMCARB, C_2MIM-HSO4 and C_2MIM-SCN. EMIM-Tf2N, HMIM-Cl and EMIM-Cl are separated to extract of Ni and VO^{+2} from

petroleum and partially deasphalted oil. DIMCARB was most efficient IL for both ions. [62]

B. Pérez et al. synthesized four TSILs; trioctyl methyl ammonium benzoate; trioctylmethyl ammonium bis-(2-ethylhexyl) phosphate; trihexyltetradecylphosphonium benzoate and trihexyltetradecylphosphonium bis-(2-ethylhexyl) phosphate; diluted in kerosene to extract Mo(VI) from aqueous solution most efficient to be [TOMA][BA] via anion exchange mechanism.[63]

S. Katsuta et al. separated gold as ion [$AuCl^-$] via ion pairing using cations 1-hexyl-3-methylimidazolium[Hmim]$^+$,1-butyl-3-methylimidazolium([BMIm]+), 1-butyl-1-methylpyrrolidinium, 1-methyl-3-octylimidazolium, methyltrioctylammonium and 1-butyl-2,3-dimethylimidazolium, the anions were BF^{4-}, hexa fluorophosphate, ([NTf2]−), and bis-(pentafluoroethanesulfonyl)amide from HCl solution into IL.[64]

M. Thayyil et al. prepared three hydrophobic ILs trihexyltetradecylphosphonium ([PC6C6C6C14]); [PC6C6C6C14][-N(CN)2−], [PC6C6C6C14][Cl^-], and [PC6C6C6C14][-NTf2−] for liquid-liquid separation of dyes, heavy metals; Cr, Hg, Zn, Pb etc and phenolic compounds from industrial water upto 100% efficiency. Phoephonium chloride RTIL was best for phenolic chemicals characterized by ICP-MS, UV-MS and thermo gravimetric analysis. [65]

M. Hosseni et al. synthesized IL clay to adsorb heavy metals from water and characterized it through various techniques of thermogravimetry, XRD, SEM and EDX. These IL clay separated zinc, cobalt and lead metals. [66]

N. Sidek and coworkers prepared reuseable [DBZIM]Cl ,1-Allyl-3-benzylimidazolium chloride [ABZIM]Cl and [BZVIM]Cl to extract phenolic substances present in oil; hexane, cyclohexane, heptane and petroleum ether and characterized by proton and carbon NMR and FTIR.[67]

Y. Ghorbani et al. extracted cadmium ion with 3,3′-(hexyl)bis(3-methylimidazolium) bromide chloride ([H(mim)2[Br][Cl]),1-hexyl-3-methylimidazolium chloride ([Hmim][Cl]) and 1-octyl-3-methylimidazolium chloride ([Omim][Cl]) IL clay modified by Na-bentonite (Bent) and montmorillonite (MT) with best separating efficiency of [Omim][Cl] through adsorption mechanism. Characterizations done were SEM, XRD, FTIR and CHN analysis. [68]

J. Alvarez-daboy et al. made 1-Butyl-3-metylimidazolium $[BMIM]^+$ based $[BMIM]^+[SCN]^-$, $[BMIM]^+[PF6]^-$ and $[BMIM]^+[Cl]^-$ ILs to separate Hg^0 from air. $[BMIM]^+[Cl]^-$,$[BMIM]^+[SCN]^-$ gave better separation examined by PIXE technique (particle induced X ray emission). [69]

X. Xiao et al. selectively separated Rhenium (VII) through adsorption using imidazolium IL modifying them with chitosan via anion exchange mechanism characterized by XRPS, ion chromatography and FTIR techniques. [70]

2.4. Research work aims:

- This research aims to control and treat bacterial infections that have spread everywhere in schools, homes, parks and hospitals.
- To investigate ionic liquid based paints that can cover and kill bacteria on the surfaces for long period of time.
- To study ionic liquids to remove toxic heavy metals from surroundings.
- To synthesize ionic liquids in the laboratory and characterize them via TLC, FTIR and NMR techniques.
- Evaluation of synthesized ionic liquids through their antibacterial efficiency and heavy metal removal.

Chapter 3

3. Experimental

This chapter includes synthesis of different ionic liquids in lab, at first step bromide type ionic liquids were synthesized then bromide anion was replaced with different anions that was:

Dioctyl succinamic acid (DOSA).

3.1 Solvents and chemicals

All solvents were dried with help of distillation assembly different solvents used were: Methanol (CH3OH), Chloroform (CHCl3), Acetonitrile (CH3CN), n-Hexane, Ethyl acetate and Distilled water.

Chemicals were: 1-Methyl imidazole, Pyridine, Imidazole, Pyrazine, 1-bromo octane, Succinic Anhydride, Dioctyl amine, Sodium hydride

3.2 Apparatus and Glassware

Weighing balance, Vacuum oven, Vacuum pump, Fume hood, Hot plates, Nitrogen cylinder, Drying oven, pH papers and pH meter, Filter paper, Rotary Evaporator, Clamps and stands, TLC plates.

Beakers, conical flasks, 2 neck and single neck Round bottom flasks, Condenser, Rubber stoppers, Magnetic stirrer, Glass vials, Reagent bottles, Distillation assembly, Spatula, Pipettes, Thermometer, Oil bath, Measuring cylinder, Nitrogen balloons, Funnel, Syringes, Ependorf tubes, Capillaries.

3.3 Experimental procedure

Different bromide ILs and anion Dioctyl succinamic acid were prepared.

3.3.1. 1-Methyl-3-octyl imidazolium bromide synthesis:

1-Methyl-3-octylimidazolium bromide was synthesized by reacting 1 Methyl imidazole (3g, 36 mmol) with octyl bromide (6.95, 36 mmol) in acetonitrile (60ml). For 24hr reflux was carried out to obtain maximum yield of product. Reaction completion was confirmed with help of TLC , solvent was rotary evaporated, reactants were decanted to obtain pure product, product obtained was further dried with heating oven for 12 hours at 80^{o}C product obtained as a yellow oily liquid with 91% yield.

FTIR: Sp^3 C-H (2924 cm^{-1}), C=C (1600 cm^{-1}), C-N (1166 cm^{-1}), C=N (1570 cm^{-1}),

^{1}H-NMR: δ 0.8 (3H, t, *J* = 4.2 Hz), 1.3 (6H, m), 1.9 (4H, m), 4.1 (3H, s), 4.37 (2H, t, *J* = 7.5 Hz), 10.43 (1H, s), 7.4 (1H, s), 7.3 (1H, s)

3.3.2. 1-Octyl imidazole synthesis:

Imidazole (2g, 30mmol) reacted with 1-bromo octane (6g, 31mmol) and two equivalent of sodium carbonate, refluxed for 24h in acetonitrile solvent to obtain 1-octyl imidazole as product.

Reaction progress was observed with TLC. Reaction mixture was evaporated in rotary evaporator and further dried in heating oven for 12h. 81% yield was obtained.

3.3.3. 1,3-Dioctyl imidazolium bromide synthesis:

1,3-dioctyl imidazolium ionic liquid was obtained by reaction of 1-octyl imidazole (1g, 5.6mmol) with 1-bromo octane under reflux in acetonitrile for 24h. TLC was done to check completion of reaction and solvent was evaporated through rotary evaporator. Product was further dried in oven

for 12h yield obtained was 80%.

FTIR: sp^3 C-H (2923 cm^{-1}), C=C (1562 $cm^{-1)}$, C=N (1463 cm^{-1}), C-N (1160 cm-1)

^{1}H-NMR: δ 0.79 (3H, t, *J* = 6.6 Hz), 1.3 (8H, m), 1.8 (2H, p), 4.4 (2H, t, *J* = 14.7 Hz), 7.67 (2H, d), 10.3 (1H, s)

3.3.4. 1-octyl pyridinium bromide synthesis:

Pyridine (1g, 10mmol) and 1-bromo octane (2g, 13mmol) were refluxed in acetonitrile solvent for 24h while checking reaction progress with TLC and rotary evaporated that gave dark brownish 1-octyl pyridinium bromide IL with 90% yield. Product was further dried in oven for 12h.

FTIR: sp^3 C-H (2925, 2853 cm^{-1}), C=N (1459 cm^{-1}), C=C (1640 cm^{-1}), C-N (1120 cm^{-1})

^{1} H-NMR: δ 0.7 (3H, t, *J* = 4.2 Hz), 1.29 (6H, m), 2.9 (2H, p), 3.7 (2H, p), 4.8 (2H, t, *J* = 7.5 Hz), 8.15 (2H, t, *J* = 6.5 Hz), 9.2 (2H, d, *J* = 1 Hz)

3.3.5. Dioctyl succinamic acid synthesis:

Succinic anhydride (1g, 10mmol) was reacted with dioctyl amine (2g, 9.9mmol) in dry THF solvent after reflux of 24h. Anion dioctyl succinamic acid was obtained as light yellow viscous liquid. Solvent was rotary evaporated and product was dried in oven for 12h. Yield obtained was 89%.

FTIR: sp^3 C-H (2923 cm^{-1}), C=O (1730 cm^{-1}), C-O (1168 cm^{-1})

^{1} H-NMR: δ 0.85 (3H, t, *J* = 6 Hz), 1.28 (9H, m), 1.4 (2H, p), 2.47 (2H, t, *J* = 1.8 Hz), 2.49 (2H, t, *J* = 1.8 Hz), 3.2 (2H, t, *J* = 9.6 Hz)

3.3.6. [1-Methyl-3-octyl imidazolium] Dioctyl succinamic acid synthesis:

In a two neck round bottom flask dioctyl succinamic acid (0.5g, 1.4mmol) was taken in 35ml dry THF. Moisture of solvent was removed by heating flask with a hot gun and vaccum creator. System was kept inert by nitrogen ballon. Afterwards flask was kept on an ice bath to get 0ºC temperature at which NaH (0.11g, 4.5mmol) was added and allowed to stir for 24h that gave sodium salt of dioctyl succinamic acid. After 24h 1-Methyl3-octyl imidazolim bromide (0.4g) was added in reaction mixture and allowed to stir for further 24h. Task specific IL (TSIL) was obtained after anion exchange of bromide with dioctyl succinamic acid anion. Solvent was rotary evaporated and filtered with dichloromethane (DCM) or chloroform to remove NaBr ppt from product. It was dried in oven for 12h and 85% yield was obtained.

FTIR: sp^3 C-H (2930 cm^{-1}), C=C (1610 cm^{-1}), C=N (1530 cm^{-1}), C-N (1420 cm^{-1}), C-O (1240 cm^{-1}), C=O (1685 cm^{-1}),

3.3.7. [1, 3-Dioctyl imidazolium] Dioctyl succinamic acid:

Dioctyl succinamic acid (0.5g, 1mmol) is taken in two neck round bottom flask with 35ml dry THF. In inert atmosphere moisture of solvent was removed by heating with hot gun while connecting with vaccum pump. On an ice bath at 0ºC NaH (0.1g, 5mmol) is added in the system and alloed to stir for 24h. Then 1,3-dioctyl imidazolium bromide(0.5g) is added and stirred for further 24h. Solvent is evaporated with rotary evaporator and filteration was done with DCM or chloroform to remove NaBr ppt. TSIL obtained in 80% yield is dried in oven for 12h.

FTIR: C –H (2922 cm^{-1}), C=O (1650 cm^{-1}), C-N (1447 cm^{-1})

3.3.8. [1-Octyl pyridinium] Dioctyl succinamic acid:

In two neck round bottom flask dioctyl succinamic acid (0.5g, 1.5mmol) is taken in 35ml dried THF in nitrogen atmosphere and removing moisture with vacuum pomp while heating with hot gun. NaH (0.1g, 4.6mmol) is added at 0ºC and stirred for 24h. 1-octyl pyridinium bromide [0.4g] is added and further stirring was done for 24h. Solvent is rotary evaporated and TSIL is obtained by filtering with DCM or chloroform to separate NaBr ppt from our product. IL is dried in oven for 12h. Yield obtained was 86%.

FTIR: sp^3 C-H (2922 cm^{-1}), C-N (1447 cm^{-1}), C=O (1650 cm^{-1}),

^{1}H-NMR: δ 0.85 (3H, t, *J* = 6 Hz), 1.28 (9H, m), 1.4 (2H, p), 2.39 (2H, t, *J* = 7.2 Hz), 2.49 (2H, t, *J* = 1.8 Hz), 4.6 (2H, t, *J* = 7.5 Hz), 8.2 (2H, t, *J* = 6.9 Hz), 8.6 (1H, t, *J* = 7.8 Hz), 9.2 (2H, d, *J* = 5.7 Hz)

Chapter 4

4. Results and Discussions

Various TSILs formed were characterized by different techniques;

- Thin Layer Chromatography
- Fourier transform Infrared Spectroscopy
- Nuclear Magnetic Resonance

4.1. Thin Layer Chromatography (TLC)

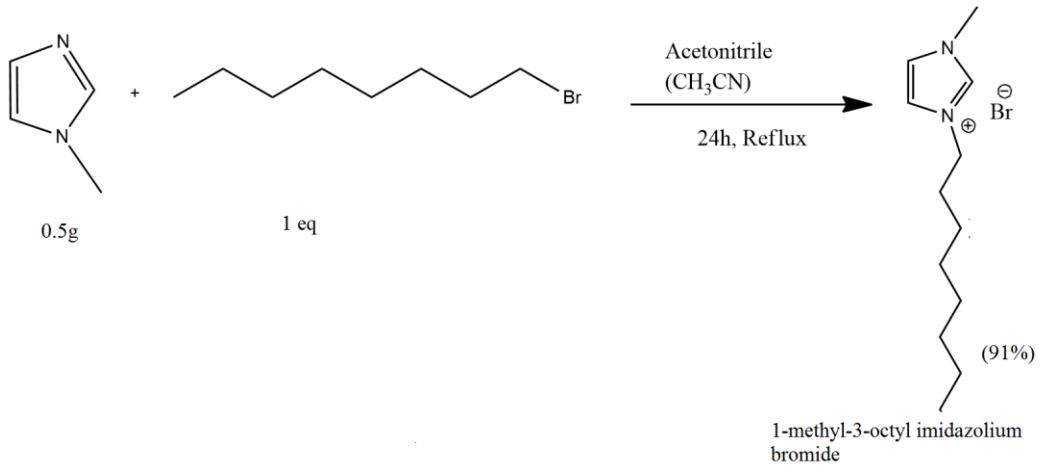
This is a chromatographic technique which give us information about purity and progress of reaction on the basis of the spot travel between stationary and mobile phase. Stationary phase in TLC is thin silica layer on plate while mobile phase may be polar or non-polar like methanol, hexane, acetic acid etc. Progress of reaction is checked from time to time until spots show completion of reaction. Invisible spots can be checked under UV lamp or indicating agents like iodine which give yellow or brown spots.

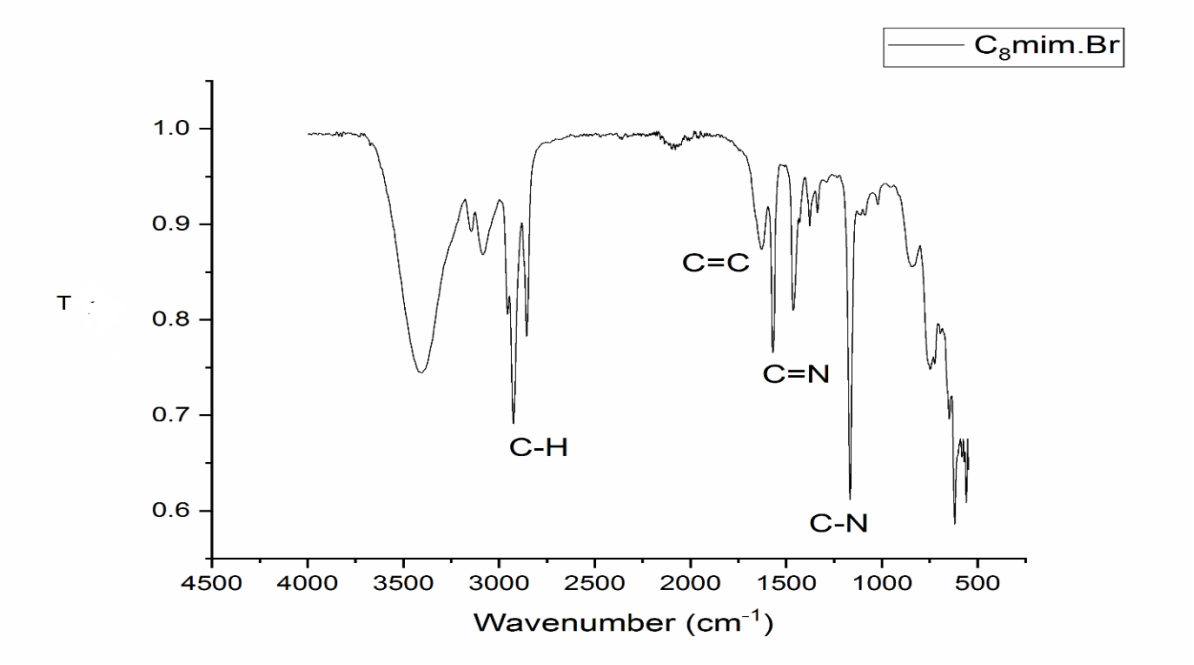
4.2. Fourier Transform Infrared Spectroscopy (FTIR)

It is characterization technique which gives indication of functional groups present in the organic compounds according to the peaks in respective regions. Different functional groups show varying vibrations which distinguish functional groups with fingerprint region lying in range of 400-4000 cm^{-1}. Vibrations may be stretching or bending.

4.2.1. 1-methyl-3-octyl imidazolium bromide:

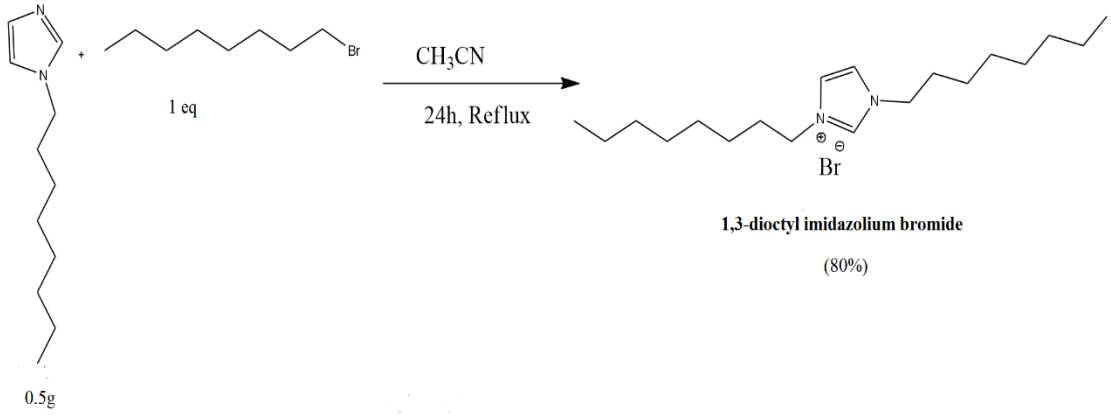
This cation is characterized by FTIR and NMR spectroscopies. FTIR of reactant methyl imidazole has no long peak of octyl chain. IR of bromo octane has no C=N, C=C and C-N stretching peaks. After reaction between two reactants new peaks appear in IR spectrum showing formation of cation through peaks of sp^3 C-H stretch and C=C, C=N and C-N stretching peaks at 2900, 1650, 1500 and 1300 cm^{-1}. Similarly ^1H- NMR spectrum show chemical shifts of protons that have both alkyl chain and imidazole protons peaks that are not present in separate methyl imidazole and bromo octane spectrums. Due to presence of bromide anion peak of protons is shifted to higher ppm value up to 4.4ppm.

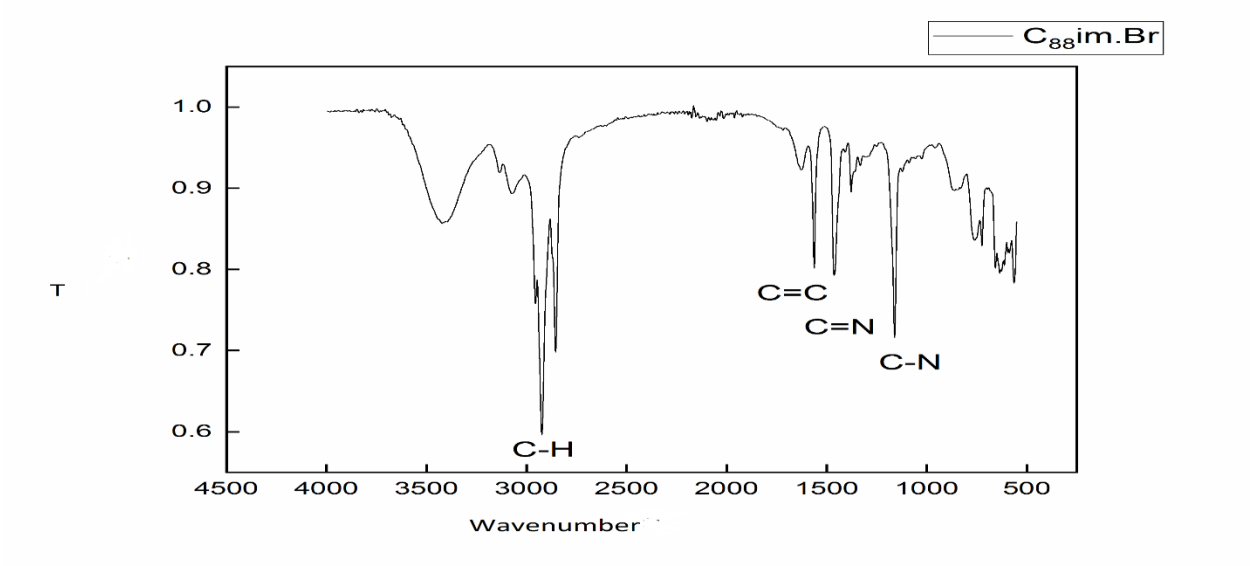




4.2.2. 1,3-dioctyl imidazolium bromide:

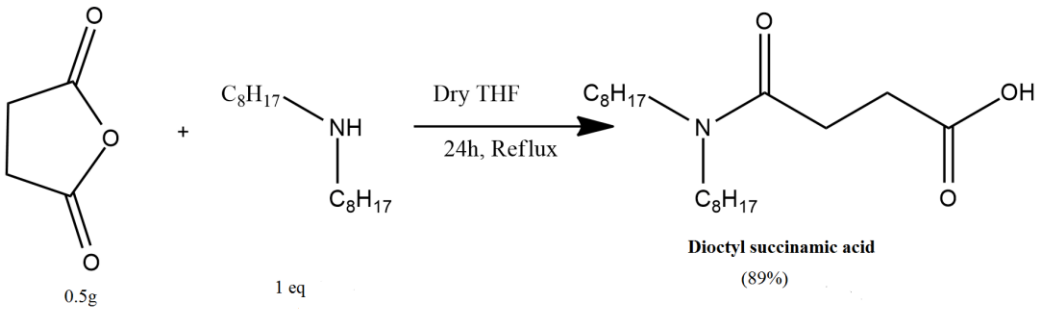
Appearance of new peaks of saturated octyl chain at 2930$cm^{-1,}$, 1540 1350 and 1659cm^{-1} for C-H, C=N, C-N and C=C peaks at in FTIR spectrum indicate formation of cation . In ^{1}H-NMR spectrum triplets, multiplets of octyl chain at 0.8, 1.3, and 4.4 ppm shows presence of CH_3, 12-H of six CH_2 and N-CH_2 respectively. Protons of imidazole appear at 7.65 and 10.3ppm.

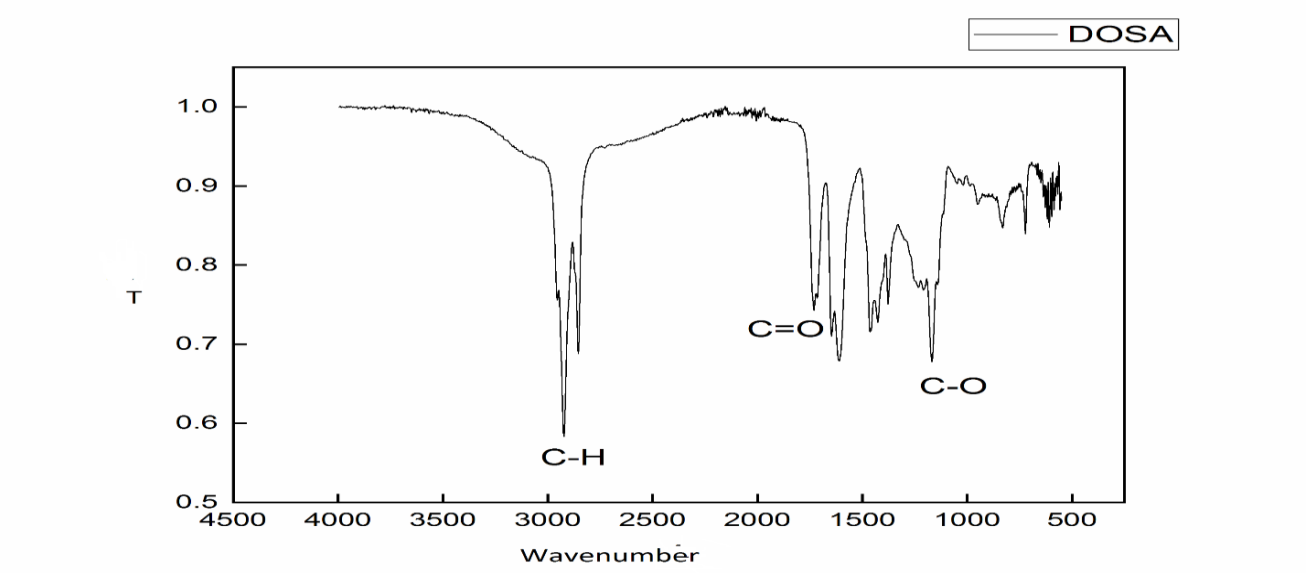




4.2.4. Dioctyl succinamic acid:

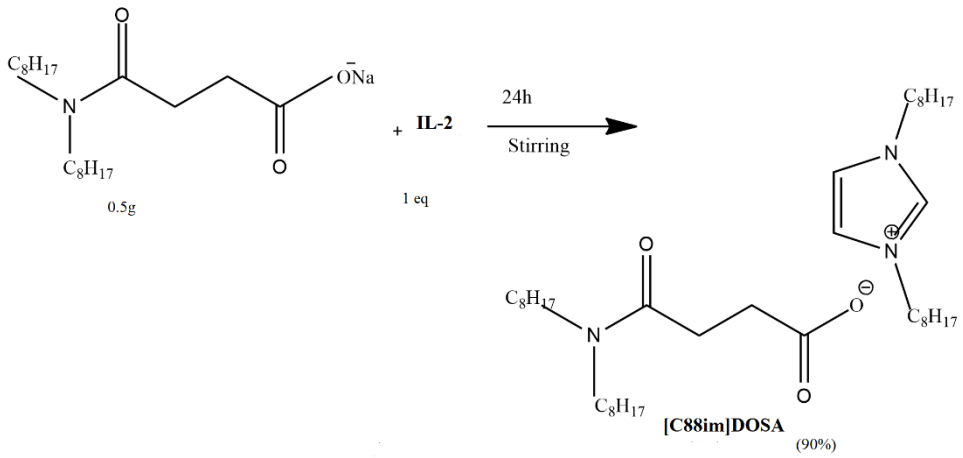
In FTIR spectrum in contrast of IR spectra of succinin acid or dioctyl amine, appearance of both carbonyl carcon C=O peak at 1700 cm^{-1} and saturated alkyl chain at around 2930 cm^{-1} in anionic spectrum shows formation of dioctyl succinamic acid. ^{1}H-NMR spectrum gives peaks of saturated alkyl chain in which the two triplets at 2.5 and 2.6 ppm indicate reaction between succinic anhydride and dioctyl amine. Other peaks appear between 0.8-4.4 ppm of the alkyl chain.

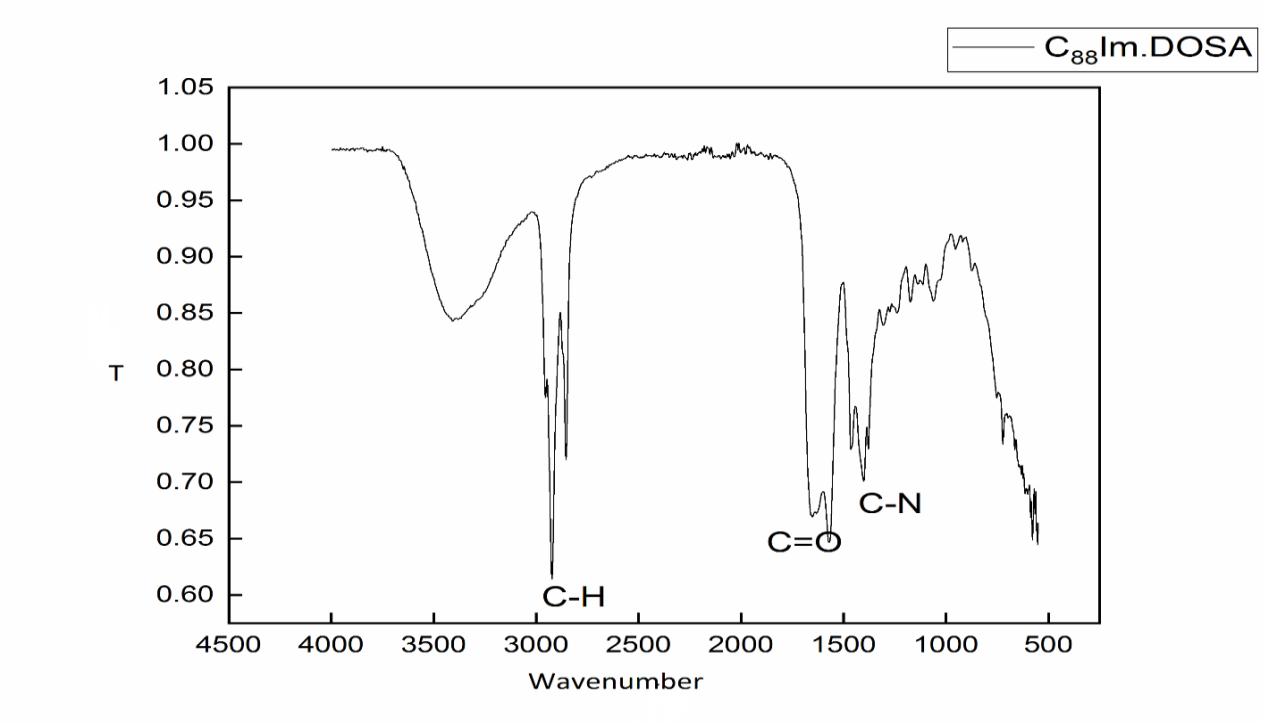




4.2.5. [1,3-dioctyl imidazolium] DOSA:

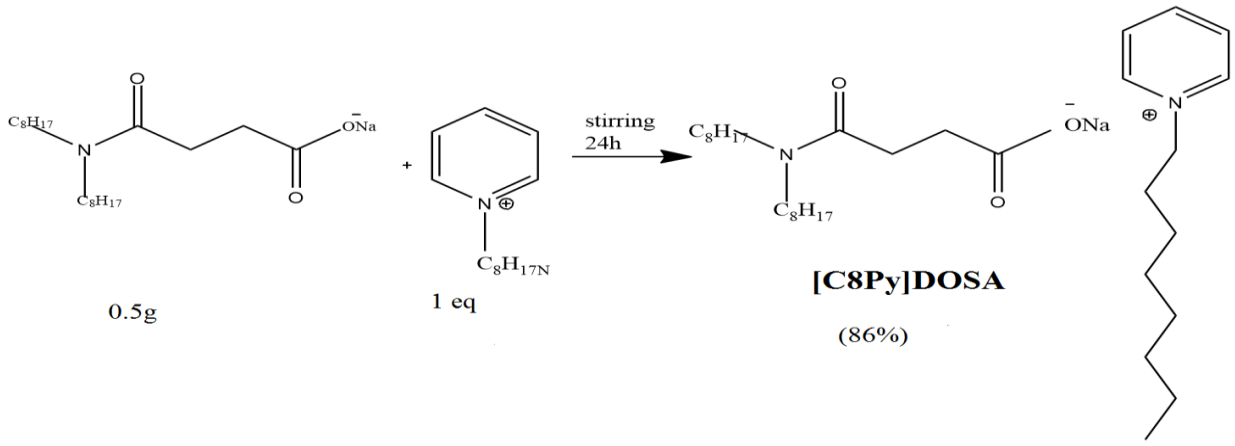
Peaks of octyl chain at 2930 cm^{-1} is not present in spectrum of imidazole reactant and peaks of C=C, C=N and C-N near 1600, 1550 and 1300 cm^{-1} respectively are not in bromo octane reactant. All peaks appearing in spectra of ionic liquid shows formation of the product. Also peak of and C-O at 1250 cm^{-1} and C=O at 1690 cm^{-1} shows DOSA anion.

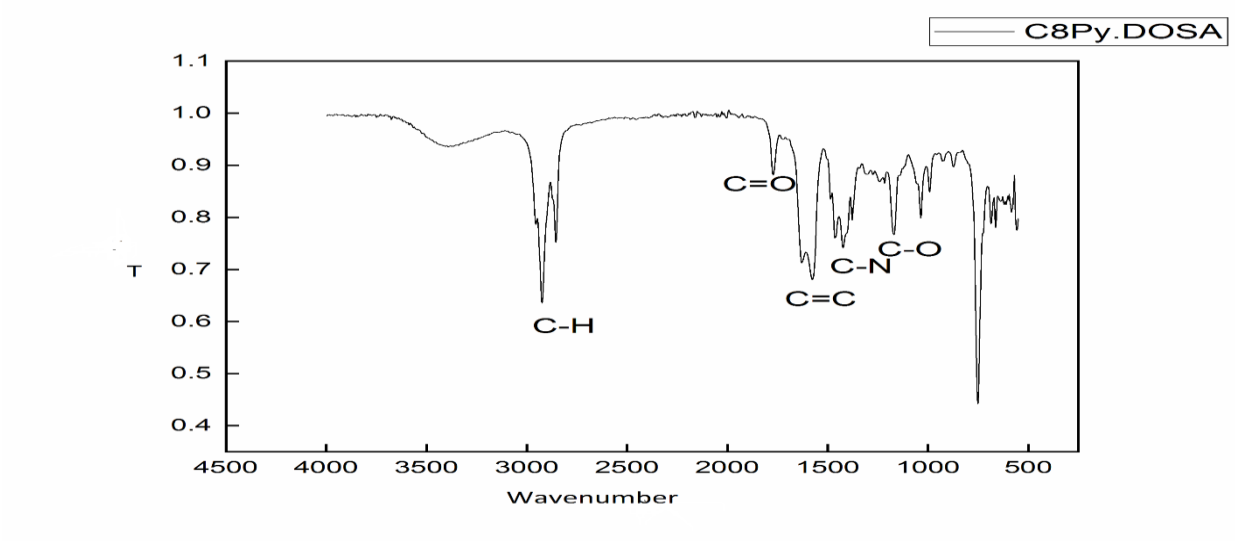




4.2.6. [1-octyl pyridinium] Dosa:

Characterization of IL with FTIR indicate formation of ionic liquid through emerging peaks of C=O, C-O, sp^3 C-H, C=C, C=N and C-N in a single spectra around 1680, 2930, 1590, 1450 and 1290 cm^{-1}. Structure is confirmed from ^{1}H-NMR spectra that gives two triplets and a doublet of pyridine protons at 8.2, 8.6 and 9.2 ppm. Anion is indicated through two triplets of four protons at 2.5 and 2.6 ppm, N-CH_2 give triplet at 4.3 ppm.

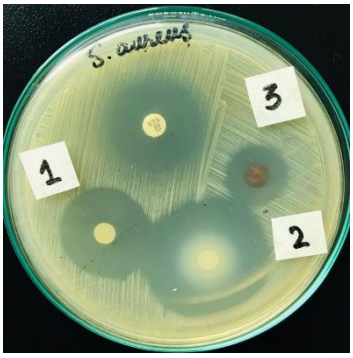

stirring
24h
0.5g
1 eq
[C8Py]DOSA
(86%)

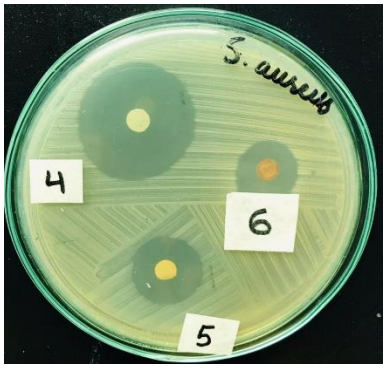

C8Py.DOSA
T
Wavenumber
C-H
C=O
C=C
C-N
C-O

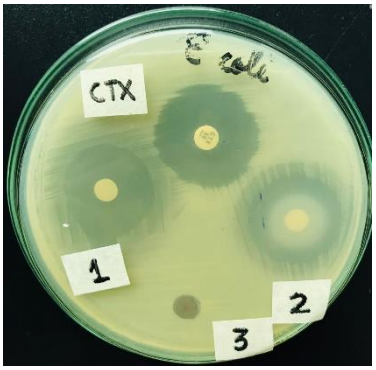
4.3. Anti-bacterial Applications

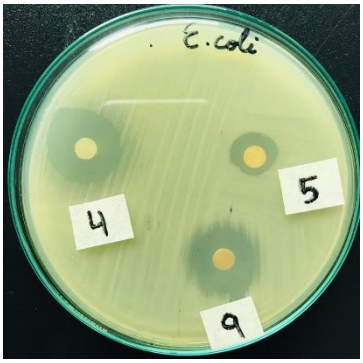
4.3.1. Agar well diffusion method:

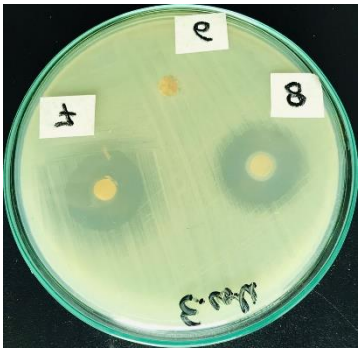
Anti-bacterial activity is determined by agar well diffusion method in which a hole is created in the agar and our compound is injected on bacteria. Zone of inhibition is measured in mm. Zone of inhibition showed by ILs are as follows;











Zones of Inhibition:

Positive control= CTX

Negative control= DMSO

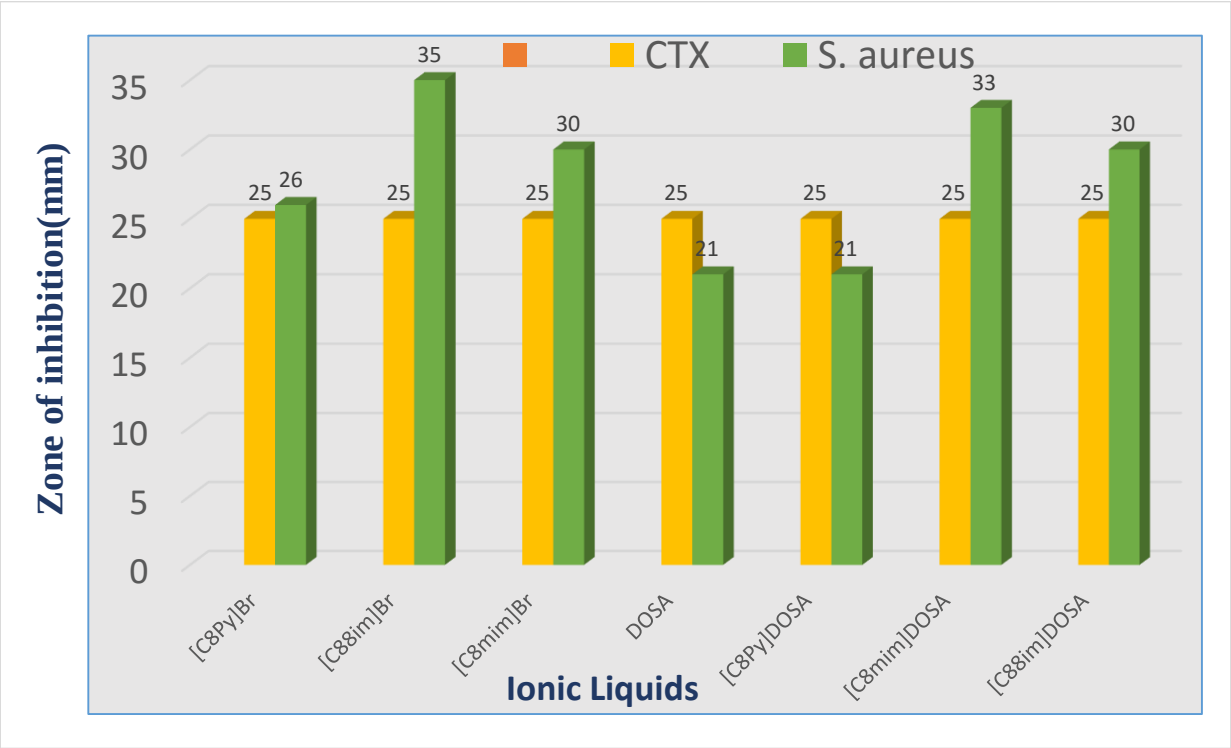
Zone of inhibition of CTX against *E. coli* is 25 mm and against *S. aureus* is 26 mm.

Table: Zones of Inhibition in mm

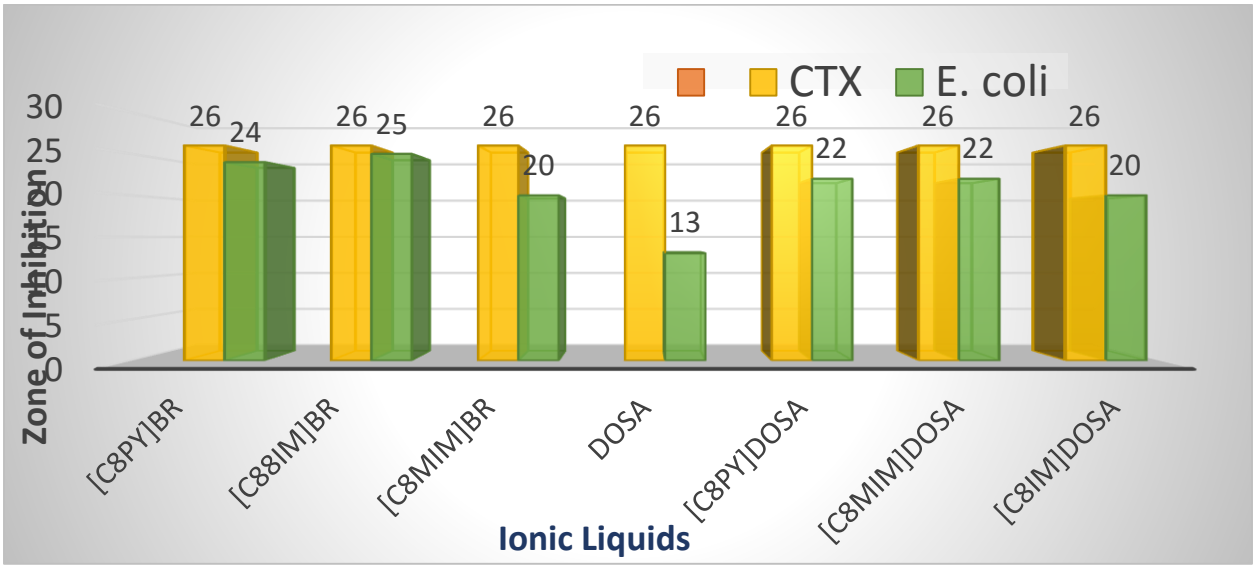
Code#	**Compounds**	***S. aureus***	***E. coli***
1.	1-octyl pyridinium bromide [C_8Py]Br	26	24
	1,3-Di-octyl Imidazolium bromide[C88Im]Br	35	25
3.	1-octyl pyrazinium Bromide [C_8Pyr]Br	19	7
4.	1-Methyl-3-octyl imidazolium Bromide [C_8mim]Br	30	20
5.	Dioctyl succinamic acid DOSA	21	13
6.	1-octyl pyrazinium Dioctyl succinamic acid (pyr.DOSA)	16	-
7.	1-octyl pyridinium Dioctyl succinamic acid (C_8pyr.DOSA)	21	22
8.	1-Methyl-3-octyl imidazolium DOSA [C8mim]DOSA	33	22
9.	1,3-Dioctyl immidazolium Dioctyl succinamic acid [C88im]DOSA	30	20

Graphical representation of zones of inhibition

For *S. aureus*



For E. coli



4.4. Conclusion:

TSILs were formed for their anti-bacterial activity for gram positive *S. aureus* and gram negative *E. coli.* Activity was good for *S. aureus* than *E. coli*. [C88im]Br, [C8mim]DOSA, [C8mim] Br and [C88im]DOSA showed better inhibition for *S. aureus* while [C88im]Br, [C8pyr]DOSA and [C8mim]DOSA were effective against *E.coli.* Hence synthesized ionic liquids exhibited as good anti-bacterial agents and can be used in anti-bacterial paints and disinfectants etc.

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