

# Novel Synthesis, Characterization and Antimicrobial Activities of Silver Nanoparticles in Room- Temperature Ionic Liquids

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**ABSTRACT** - Stable silver nanoparticles were successfully synthesized by chemical reduction of silver nitrate in ionic liquids 1-Ethyl-3-methylimidazolium tetrafluoroborate [Emim] BF<sub>4</sub> and 1-Ethyl-3-methylimidazolium hexafluorophosphate [Emim] PF<sub>6</sub> at room temperature. The characterization of the silver nanoparticles such as their size and shape was performed by X-ray Diffraction (XRD) and Scanning Electron Microscope (SEM) techniques which indicated a size range of 50 to 55 nm. The antimicrobial activity of silver nanoparticles for three gram negative bacteria and three gram positive bacteria were investigated. It appeared that [Emim] PF<sub>6</sub> and its Ag nanoparticles are the most effective products against the tested bacterial stains compared with [Emim] BF<sub>4</sub> and its Ag nanoparticles.

**Keywords:** silver nanoparticles, ionic liquids, gram negative bacteria, gram positive bacteria, antimicrobial activity, scanning electron microscopy, X-ray Diffraction.

## 1. INTRODUCTION

The development of cleaner technologies is a major emphasis in green chemistry. Among the several aspects of green chemistry, the reduction/replacement of volatile organic solvents from the reaction medium is of utmost importance. The use of a large excess of conventional volatile solvents required to conduct a chemical reaction creates ecological and economic concerns. The search for a nonvolatile and recyclable alternative is thus holding a key role in this field of research. The use of fused organic salts, consisting of ions, is now emerging as a possible alternative. A proper choice of cations and anions is required to achieve ionic salts that are liquids at room temperature and are appropriately termed room temperature ionic liquids (RTILs). Common RTILs consist of N,N'-dialkylimidazolium, alkylammonium, alkylphosphonium or N-alkylimidazolium as cations [1]. Most of these ionic salts are good solvents for a wide range of organic and inorganic materials and are stable enough to air, moisture, and heat. Ionic liquids are polar (but consist of poorly coordinating ions), and immiscible with a number of organic solvents, and therefore provide polar alternatives for biphasic systems.

Aqueous mediated reactions offer useful and more environmentally friendly alternatives to their harmful organic solvent versions and have received increasing interest in recent years. Furthermore, water has unique physical and chemical properties, and by its utilization it would be possible to realize reactivity or selectivity that cannot be attained in organic solvents. Water is the most abundant, cheapest, and non-toxic chemical in nature. It has high dielectric constant and cohesive energy density compared to organic solvents. It has also special effects on reactions arising from inter and intramolecular non-covalent interactions leading to novel solvation and assembly processes. Water as a reaction medium has been utilized for large numbers of organic reactions [2].

Room-temperature ionic liquids (RTILs) have attracted intensive interests only in recent years as a replacement for classical molecular solvents in fundamental researches and application including separation, catalysis, organic synthesis, and so on [3].

Nanoparticles have been extensively investigated due to the attraction of their unique physical properties, chemical reactivity, and potential applications with high academic and industrial impacts [4]. Silver nanoparticles may have an important advantage over conventional antibiotics in that it kills all pathogenic microorganisms, and no organism has ever been reported to readily develop resistance to it. Researchers believe that the potential of colloidal silver is just beginning to be discovered.

In the present an attempt has been made to synthesize silver nanoparticles in ionic liquid [Emim] BF<sub>4</sub> and [Emim] PF<sub>6</sub> with sodium citrate as a reducing agent. Effects of the reduction temperature and the precursor concentration on the size of the silver nanoparticles were investigated. The silver nanoparticles synthesized in this method were characterized by XRD and SEM analyses and their antimicrobial activities were screened for three gram (+) and three gram (-) bacteria.

## 2. EXPERIMENTAL

### 2.1 Materials

All chemicals were of AR grade. They were purchased from Merk, SD Fine Chemicals Limited and used without further purification. All the solvents and reagents were used as received and all reactions were run in oven-dried glassware. The homogeneity of the products was checked on TLC plates coated with silica gel-G and visualized by exposure to iodine vapors.

## 2.2 Instruments

The  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were recorded in  $\text{CDCl}_3$  and  $\text{DMSO-d}_6$  on a Jeol JNN ECX 400P spectrometer. The IR spectra were obtained on a Varian 800 FT-IR as thin films or for solid samples. Nanoparticles were well characterized by powder X-ray diffraction (powder XRD) and Scanning Electron Microscope (SEM). The phase, purity and crystalline size of the Silver nanoparticles were studied by XRD. Based upon the peak broadening in the patterns indicates that the Silver nanoparticles were very small in size. In addition to identification of the crystalline phases, the XRD data were used to estimate the size of the constituent crystallites by scherrer's equation. The average particle size,  $D$  was determined by Eq.  $D = K\lambda / (\beta \cdot \cos\theta)$ . Where  $\lambda$  is the wavelength of X-ray radiation (0.15406),  $K$  the scherrer's constant ( $K = 0.9$ ),  $\theta$  the characteristic X-ray radiation and  $\beta$  is full width at half maximum of the plane. The X-ray diffraction (XRD) patterns were recorded on a Philips Xpert X-ray diffractometer with  $\text{Cu K}\alpha$  radiation ( $\lambda = 0.15406 \text{ nm}$ ) employing a scan rate of  $1^\circ/\text{min}$  in the  $2\theta$  range from  $20^\circ$  to  $80^\circ$ . Surface morphology and the distribution of particles were characterized by a LEO 1430VP scanning electron microscopy (SEM) using an accelerating voltage of 15 kV.

## 2.3 Synthesis of ionic liquid [Emim] Br [5]

1-Methylimidazole (41.0 g, 0.5 mol) was added drop wise to bromoethane (54.5 g, 0.5 mol) in a 500 mL three-neck round-bottom flask equipped with a reflux condenser and a magnetic stirrer, and cooled in an ice-bath, as the reaction is highly exothermic. Having been vigorously stirred for 5 h, the mixture was refluxed in room temperature until it turned into solid completely. The solid was pounded to pieces and washed four times, each with 50 mL trichloroethane. Then the product obtained [Emim]Br (87.9 g), was dried under vacuum at  $70^\circ\text{C}$  for 24 h and it was characterized with FT-IR (neat): 3,155, 3,105, 2,927, 2,857, 1,572, 1,460, 1,169, 837, 753,  $620 \text{ cm}^{-1}$ ;  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.66 (s, 1H), 7.42 (t, 1H), 7.18 (t, 1H), 4.11 (t, 2H), 3.79 (s, 3H), and 1.27 (t, 3H).  $^{13}\text{C-NMR}$  (75 MHz)  $\delta$  = 136.24, 123.59, 121.94, 44.91, 36.42, 15.46.

## 2.4 Synthesis of ionic liquid [Emim] $\text{BF}_4$ [6]

To a solution of crude imidazolium bromide (19.2g, 0.1mol) obtained from the above reactions, in acetone (70 mL) was added sodium tetrafluoroborate (10.90 g, 0.1mol). The reaction mixture was stirred for 24 h at room temperature. The resulting mixture was filtered through a pad of aluminum oxide to remove the sodium salt and color. Evaporation of the solvent under reduced pressure afforded the corresponding imidazolium tetrafluoroborate.

FT-IR (neat): 3,153, 3,102, 2,990, 2,878, 2,833, 2,078, 1,633, 1,571, 1,457, 1388, 1301, 1,022, 842, 760,  $619, 523 \text{ cm}^{-1}$ ;  $^1\text{H NMR}$ :  $\delta$  1.41 (t,  $J = 7.3$ , 3H), 3.85 (s, 3H), 4.14 (q,  $J = 7.3$ , 2H), 7.35 (s, 1H), 7.41 (s, 1H), 8.55 (s, 1H);  $^{13}\text{C NMR}$ :  $\delta$  15.2, 36.0, 45.1, 122.2, 123.8, 135.9.

## 2.5 Synthesis of ionic liquid [Emim] $\text{BF}_6$ [1]

In a typical synthesis of 1-ethyl-3-methylimidazolium hexafluorophosphate, EMIMBr (19.2g, 0.1mol) was transferred to round bottom flask followed by the addition of 40 ml deionized water. An aqueous solution of 65%  $\text{KPF}_6$  in a 1:1:1 molar ratio was added slowly to minimize the amount of heat generated. As  $\text{KPF}_6$  was added, two phases were formed in which [EMIM]  $\text{PF}_6$  occupied the bottom phase and KCl, the upper phase. The upper phase was decanted and the remaining product was washed with water several times. Then the resulting product was dried at  $70^\circ\text{C}$  in vacuum line for 4 h to get the desired product. FT-IR (neat): 3,175, 3,132, 2,987, 2,888, 1,615, 1,578, 1,466, 1342, 1,293, 1,174, 835, 752,  $644, 557, 433 \text{ cm}^{-1}$ ;  $^1\text{H NMR}$ :  $\delta$  1.411 (t,  $J = 7.3$ , 3H), 3.852 (s, 3H), 4.14 (q,  $J = 7.3$ , 2H), 7.648 (s, 1H), 7.727 (s, 1H), 9.068 (s, 1H);  $^{13}\text{C NMR}$ :  $\delta$  15.2, 36.0, 45.1, 122.2, 123.8, 135.9.

## 2.6 Synthesis of Silver Nanoparticles [7]

Two solutions of silver nitrate with a concentration of 0.03 mol/L and sodium citrate with a concentration of 0.02—0.05 mol/L in ionic liquid were prepared. The sodium citrate solution was then added drop wise into the  $\text{AgNO}_3$  solution under a vigorous stirring at a given temperature in the range of  $25$ — $40^\circ\text{C}$ . The addition process was conducted for 0.5 h and the solution was continuously stirred for another 3 h. After adjusting the pH at 8.0, the brown colloid contained silver nanoparticles could be formed. The products were separated by centrifugation, washed with absolute ethanol for several times, then vacuum-dried at  $60^\circ\text{C}$  for 48 h for further characterization.

## 2.7. Antimicrobial Activity (Broth dilution assay) [8]

A series of fifteen test tubes were filled with 0.5 ml sterilized nutrient broth. Sequentially, test tubes 2 – 14 received an additional 0.5 ml of the sample serially diluted to create a concentration sequence from 500 – 0.06 µg. The first test tube served as the control. All the test tubes received 0.5 ml of inoculums. The test tubes were vortexed well and incubated for 24 h at 37°C. The resulting turbidity was observed, and after 24h the Minimum Inhibition Concentration (MIC) was determined where growth was no longer visible by assessment of turbidity by optical density readings at 600nm.

### 3. RESULTS AND DISCUSSION

#### 3.1. FTIR Spectra

FT-IR spectra of pure [EMIM] BF<sub>4</sub> and Ag (0) in [EMIM] BF<sub>4</sub> ionic liquid solutions are presented in Figures 1 and 2 and the main frequencies of peaks are listed in Table 1. In Figure 1, the bands at 3153 and 3102 cm<sup>-1</sup> are assigned to the C-H of imidazole ring stretching vibration. The bands at 2990 - 2878 and 1633 cm<sup>-1</sup> are due to the stretching vibration for the C-H bands of alkyl chains and C=C groups, respectively. The bands at 1571 and 1457 cm<sup>-1</sup> are due to the imidazole ring skeleton stretching vibration. The bands at 1301 and 1022 cm<sup>-1</sup> are due to the C-H of the imidazole ring in plane deformation vibration and the stretching vibration, respectively. The bands at 842 and 760 cm<sup>-1</sup> probably originate from m-substituted imidazole ring. Compared with the pure [EMIM] BF<sub>4</sub>, several significant changes are observed in the FTIR spectra of the Ag (0) in [EMIM] BF<sub>4</sub> solution (i) Two C-H of imidazole ring stretching vibration bands are up-shifted by 7 and 16 cm<sup>-1</sup>. (ii) There is no stretching vibration for the C-H bands of alkyl chains and C=C group, there is a change in the imidazole ring skeleton stretching vibration, C-H of the imidazole ring in plane deformation vibration and the stretching vibration, in Ag (0) in [EMIM]BF<sub>4</sub>. Figures 3 and 4 displays the FT-IR spectra of pure [EMIM] PF<sub>6</sub> and Ag (0) in [EMIM] PF<sub>6</sub>, respectively. The above changes of bands demonstrate that Ag (0) has an effect on the electron cloud density of imidazole ring. Based on the analysis of FT-IR spectra, it is concluded that there are strong interactions between RTILs and AgNO<sub>3</sub>, and the interactions focus on the imidazole ring of RTILs. Present findings showed similarity to the results previously reported [9].

#### 3.2. XRD analysis

The phase, purity and crystallite size of the Ag nanoparticles were studied by XRD (Figures 5 and 6). The typical diffraction pattern shows that the Ag nanoparticle prepared with two different ionic liquids are crystalline in nature, high purity and free of impurities. The crystallite size of the Ag nanoparicles for [EMIM] BF<sub>4</sub> and [EMIM] PF<sub>6</sub> was determined from the most intense diffraction peak (101) by using the Debye-Scherrer's equation was 51 and 55 nm, respectively. The relative intensity of the diffraction peaks of Ag nanoparticle prepared with different ionic liquids deviates from one to one, suggesting that an each ionic liquid has different nanostructures in certain direction of the growing material.

#### 3.3. SEM analysis

The morphologies and dispersity of synthesized nanostructured Ag nanoparticles from ionic liquids are shown in Figures 7 and 8. It can be seen from the SEM images that the morphology of Ag nanoparticles from [EMIM] BF<sub>4</sub> exhibits the well-defined Ag nanostructure composed of nanosized, regular and uniform spherical shaped particles. Figure 7 represents silver nanoparticles with an average particle size of 64 nm. The synthesized Ag nanoparticles from [EMIM] PF<sub>6</sub> also exhibit the morphology of spherical shaped particles (Figure 8). The mean diameter read from the nanoscale bar of SEM images of Ag nanoparticle prepared from [EMIM] PF<sub>6</sub> is around 70 nm which is higher than the Ag nanostructure produced from [EMIM] BF<sub>4</sub>. SEM analysis clearly indicates that the different characteristic ionic liquids produced the Ag nanoparticles with well-defined and extended ordered morphology without any agglomeration and aggregation. The average size of Ag nanostructures received from the ionic liquids [EMIM] PF<sub>6</sub> is greater than [EMIM] BF<sub>4</sub>.

#### 3.4. Antimicrobial Activities

A preliminary investigation on the antibacterial activities of pure ILs and nanoparticles were performed through measurements of minimal inhibitory concentrations (MIC) expressed in µg/mL based on the previous study [10]. The efficiency of Ag nanoparticles stabilized by ILs were evaluated against bacterial strains through measurements of minimal inhibitory concentrations (MIC) expressed in µg/mL. The values after one day of exposure are shown in Table 2.

Six microorganisms were chosen as test strains: For three gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Aeromonas hydrophila*) and three gram positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus* and *Bacillus cereus*).

In view of the results, it appeared that [EMIM] PF<sub>6</sub> and its nanoparticle are the most effective products against the tested bacterial stains compared with [EMIM] BF<sub>4</sub> and its nanoparticles. The decreasing orders of antimicrobial activities are listed below.

*Staphylococcus aureus* : 2 = 2a > 1 > 1a  
*Micrococcus luteus* : 2 > 2a > 1a > 1  
*Bacillus cereus* : 2 > 2a > 1a = 1  
*Escherichia coli* : 2 > 2a > 1 > 1a  
*Pseudomonas aeruginosa* : 2a > 2 > 1 = 1a  
*Aeromonas hydrophila* : 2a > 2 > 1 > 1a

1. [EMIM] BF<sub>4</sub>; 1a – Ag (0) of [EMIM] BF<sub>4</sub>; 2 - [EMIM] PF<sub>6</sub>; 2a – Ag (0) of [EMIM] PF<sub>6</sub>

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## REFERENCES:

- [1] Welton T., "Room-Temperature Ionic Liquids. Solvents for Synthesis and Catalysis". Chemical Reviews, **99**, 2071, 1999.
  - [2] Naik S., Bhattacharjya G., Talukdar B. and Patel B.K, "Chemoselective Acylation of Amines in Aqueous Media". European Journal of Organic Chemistry, **2004(6)**, 1254, 2004.
  - [3] Dzyuba S. V. and Bartsch R.A, "Recent Advances in Applications of Room-Temperature Ionic Liquid/Supercritical CO<sub>2</sub> Systems". Angewandte Chemie International Edition, **42**, 148, 2003.
  - [4] Quake S.R. and Scherer A, "From Micro- to Nanofabrication with Soft Materials". Science, **290**, 1536, 2000.
  - [5] Elliot Ennis and Handy S.T, "Facile Route to C2-Substituted Imidazolium Ionic Liquids". Molecules, **14**, 2235, 2009.
  - [6] Min G.H., Yim T., Lee H.Y., Huh D.H., Lee E., Mun J., Oh S.M. and Kim Y.G, "Synthesis and Properties of Ionic Liquids: Imidazolium Tetrafluoroborates with Unsaturated Side Chains". Bulletin- Korean Chemical Society, **27(6)**, 847, 2006.
  - [7] Jing A.N., De-song W. and Xiao-yan Y, "Synthesis of Stable Silver Nanoparticles with Antimicrobial Activities in Room-temperature Ionic Liquids". Chemical Research in Chinese Universities, **25(4)**, 421, 2009.
  - [8] Canillac N. and Mourey A, "Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus aureus* and coliform bacteria". Food Microbiology, **18 (3)**, 261, 2001.
  - [9] Zhu J., Shen Y., Xie A., Qiu L., Zhang Q., Zhang S, "Photoinduced synthesis of anisotropic gold nanoparticles in room-temperature ionic liquid". Journal of Physical Chemistry C, **111**, 7629, 2007.
- Demberehnyamba D., Kim K.S., Choi S., Park S.Y., Lee H., Kim S.J., Yoo I.D, "Synthesis and antimicrobial properties of imidazolium and pyrrolidinium salts". Bioorganic & Medicinal Chemistry, **12**, 853, 2004

**Table1. Frequencies of FTIR Absorption Bands for the Pure [EMIM] BF<sub>4</sub> and Ag (0) in [EMIM] BF<sub>4</sub>, Pure [EMIM] PF<sub>6</sub> and Ag (0) in [EMIM] PF<sub>6</sub>**

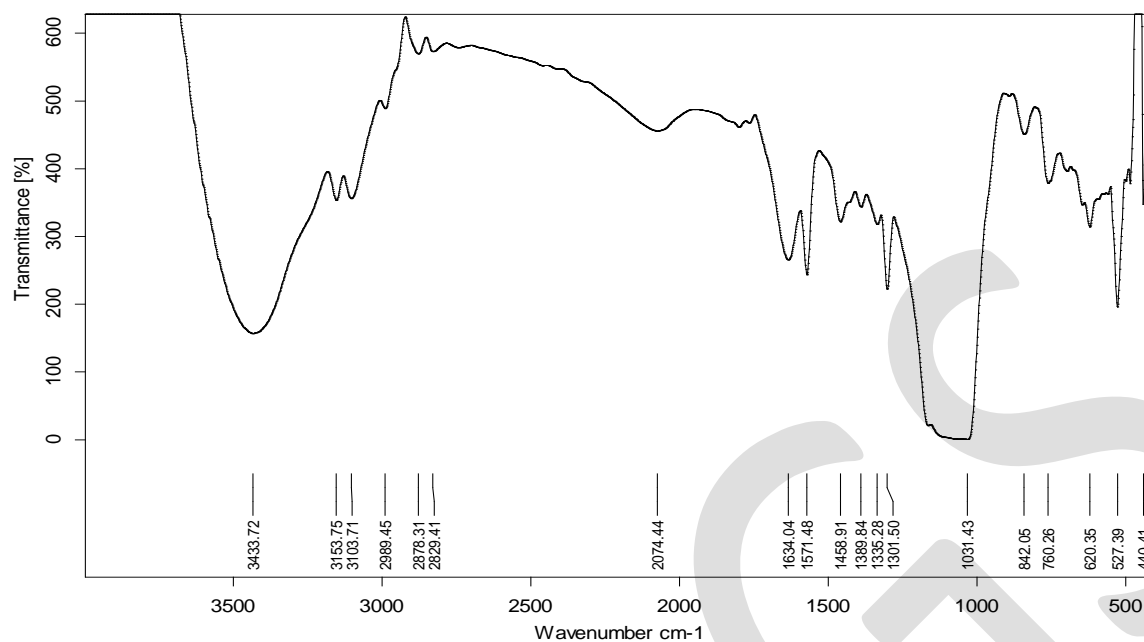
Pure [EMIM] BF <sub>4</sub>	Ag (0) in [EMIM] BF <sub>4</sub>	Pure [EMIM] PF <sub>6</sub>	Ag (0) in [EMIM] PF <sub>6</sub>	Assignments
3153, 2102	3160, 3118	3175, 3132	3173, 3124	C-H of imidazole ring stretching vibration
2990, 2833, 2078	135, 1742	2987,2888	2979, 2312, 2312	C-H of alkyl chain stretching vibration
1633	1647	1615	1647	C=C stretching vibration
1571, 1457	1518, 1462, 1423	1578, 146, 1400	1517, 1462, 1424	imidazole ring skeleton stretching vibration
1301	1166	1342, 1293	1338	C-H imidazole ring in plane deformation vibration
1022	1052	1025	1167	Stretching vibration
842, 760	727	835,752	827, 622	m-substituted imidazole ring

**Table 2. The MIC of silver nanoparticles solution stabilized by ionic liquids**

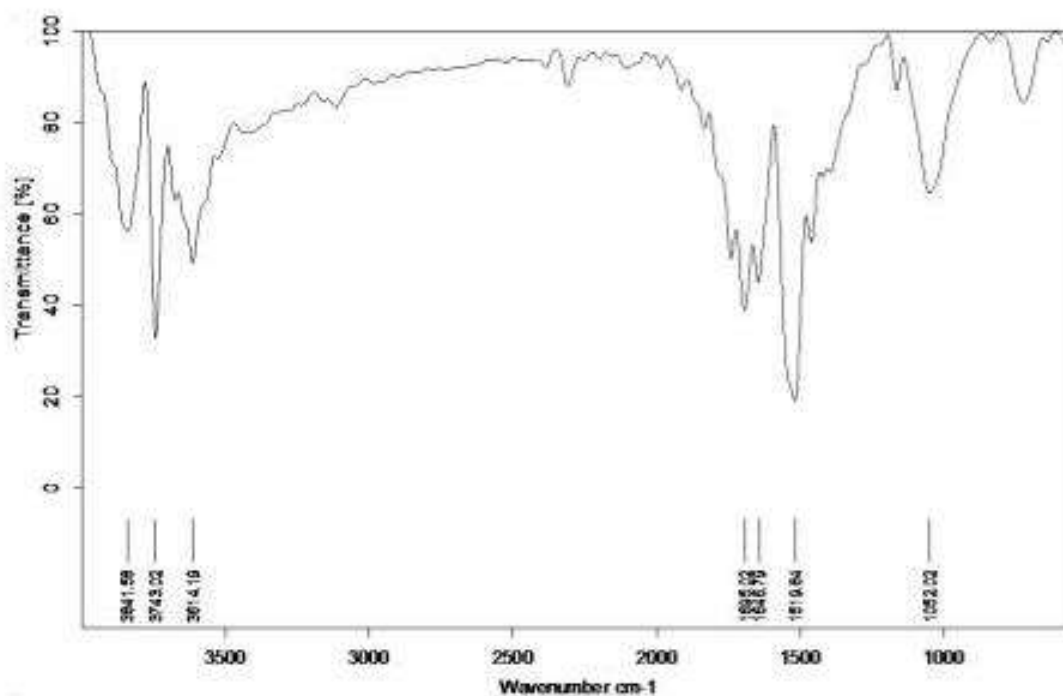
Compound number	Compound	MIC (µg/mL)					
		Tested organisms (bacteria )					
		SA	ML	BC	EC	PA	AH
1	[EMIM] BF <sub>4</sub>	31.25	15.63	15.63	3.91	3.91	31.25
1a	Ag (0) in [EMIM] BF <sub>4</sub>	250	7.81	15.63	250	3.91	125
2	[EMIM] PF <sub>6</sub>	3.91	1.95	1.95	0.98	15.63	15.63
2a	Ag (0) in [EMIM] PF <sub>6</sub>	3.91	3.91	3.91	3.91	0.98	3.91

SA - *Staphylococcus aureus*; ML - *Micrococcus luteus*; BC - *Bacillus cereus*; EC - *Escherichia coli*; PA - *Pseudomonas aeruginosa* and AH - *Aeromonas hydrophila*

**Figure 1: FTIR spectra of synthesized [EMIM] BF<sub>4</sub> ionic liquid**



**Figure 2: FTIR spectra of synthesized Ag (0) in [EMIM] BF<sub>4</sub> ionic liquid**



**Figure 3: FTIR spectra of synthesized [EMIM] PF<sub>6</sub> ionic liquid**

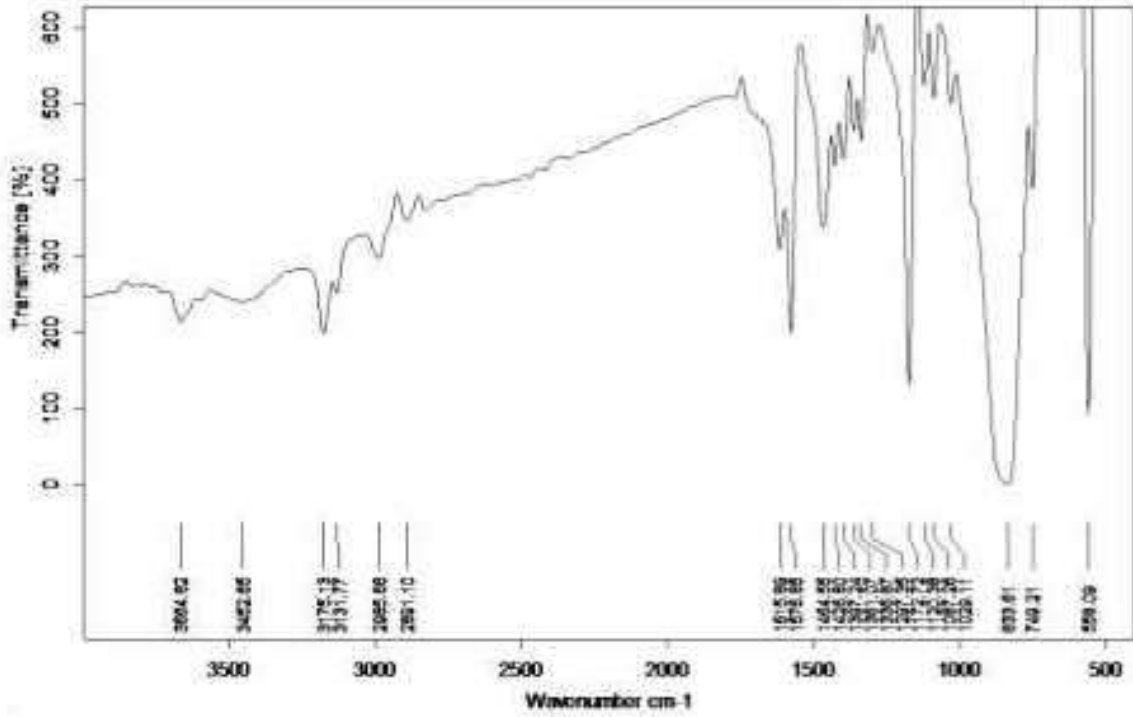


Figure 4: FTIR spectra of synthesized Ag (0) in [EMIM] PF<sub>6</sub> ionic liquid

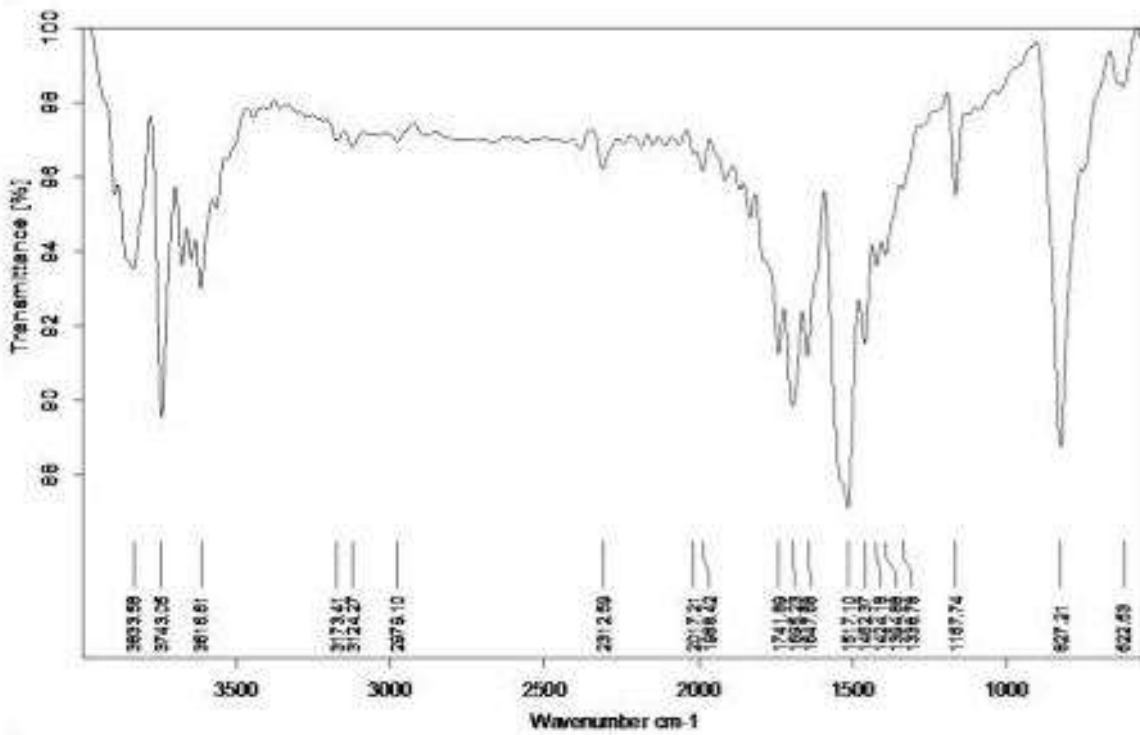
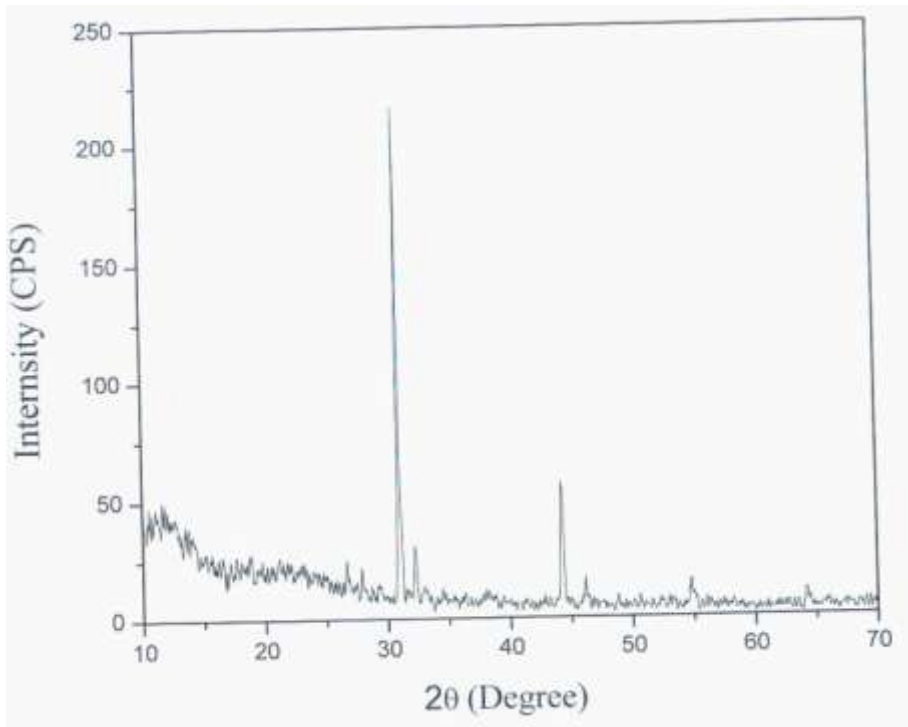
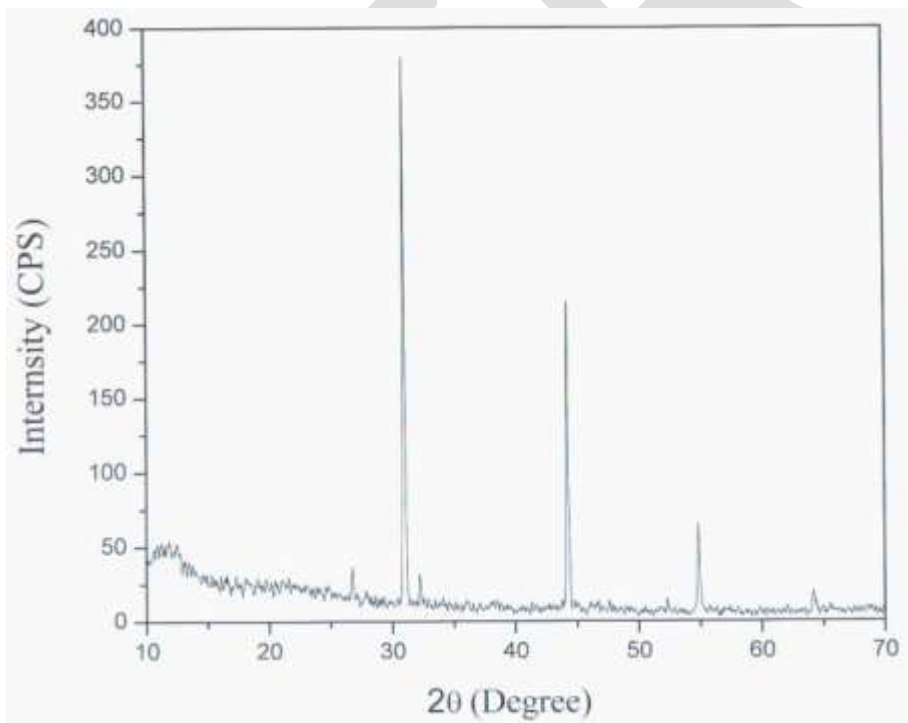


Figure 5: XRD Pattern for Ag (0) in [EMIM] BF<sub>4</sub> ionic liquid

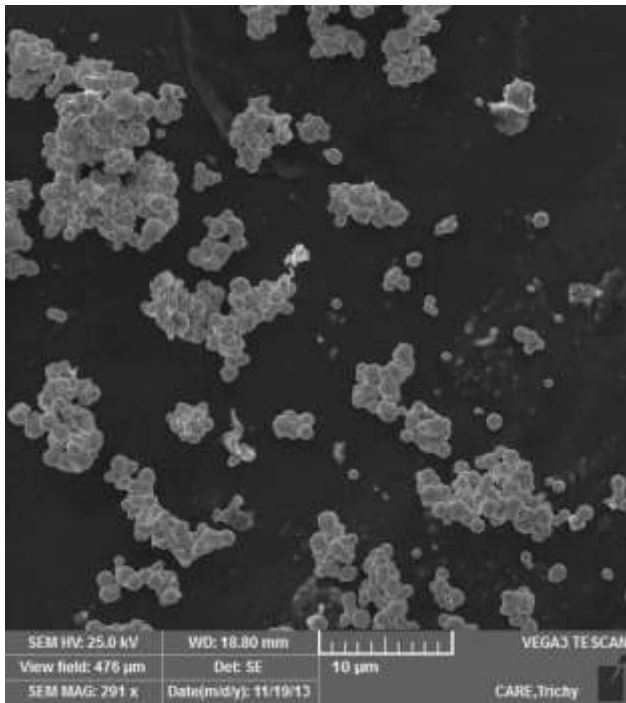


**Figure 6: XRD Pattern for Ag (0) in [EMIM] PF<sub>6</sub> ionic liquid**



**Figure 7: The SEM image for Ag (0) in [EMIM] BF<sub>4</sub> ionic liquid**





**Figure 8: The SEM image for Ag (0) in [EMIM] PF<sub>6</sub> ionic liquid**

