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Fabrication of silver nanoparticles via self-regulated reduction by 1-(2-hydroxyethyl)-3-methylimidazolium tetrafluoroborate

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Abstract−Silver nanoparticles exhibiting antimicrobial properties via self-regulated reduction were successfully prepared by using hydroxylated ionic liquids in an aqueous phase without additives. A new water-phase synthesis of silver nanoparticles using 1-(2-hydroxyethyl)-3-methylimidazolium tetrafluoroborate ([HEMIm][BF_4]) and 1-(2′-hydroxyethyl)-2-methyl-3-dodecylimidazolium chloride ([C_{12}HEMIm][Cl]) was described. 1-(2-hydroxyethyl)-3-methylimidazolium tetrafluoroborate serves as both a reductant and a stabilizer in this fabrication. Furthermore, we presented the antimicrobial properties of the resulting silver nanoparticles through the minimal inhibitory concentrations (MIC) test.

Key words: Ionic Liquid, Silver Nanoparticles, Antimicrobial Properties

INTRODUCTION

Ionic Liquids (ILs) have been mainly considered as new reaction media, as catalysts, and as electrolytes for various battery systems because of their unique electrochemical and physical properties [1,2]. Recently, considerable researches on nano-materials have been attempted on Room Temperature Ionic Liquids (RTILs) because RTILs generally feature a good stability to air and water, a wide liquid range, and unique solubility properties to various organic and inorganic substances [3-5]. Moreover, both the length of the side chain of the cation and the specifically designed anion can be easily controlled for various uses. These desirable characteristics facilitate the application of ILs to the stabilizers for preparing metal nanoparticles.

When metal nanoparticles are prepared by chemical methods, the metal ions are reduced with the reducing agents and the nanoparticles are stabilized with the protective agents such as polymers, surfactants, and thiols preventing aggregation by using electrostatic or steric means [6]. However, it has been difficult to choose a reaction medium solvent according to the species of the stabilizer. Furthermore, though several types of reducing agents, such as $NaBEt_3H$, $LiBEt_3H$, and $NaBH_4$, play a key role in determining the nucleation rate and particle size, the resulting metal nanoparticles can easily become contaminated with borides [4,5,7] In particular, the water-phase preparation of boride-free metal nanoparticles is required for biological and medical purposes.

This study focuses on a novel silver (Ag) nanoparticle stabilized by hydroxylated ILs (HILs). Ag nanoparticles have a long history of use in medicine owing to their antimicrobial properties. However, reports on the preparation and characterizations of Ag nanoballs have been limited compared to those on gold, palladium, and platinum nanoparticles. In our recent publication, we investigated the size-selective synthesis of gold and platinum nanoparticles using thiol-functionalized ionic liquids (TFILs) as stabilizers [4,5]. However, though our suggested synthesis based on TFILs has some useful advantages, challenges still remain. TFIL synthesis is complicated because of its multi-step synthesis procedures, and it is unpleasant because of the odor of raw materials. Also, during the preparation of metal nanoparticles, an expensive and toxic reducing agent must be employed to reduce the metal ions. Therefore, here, we describe a new water-phase synthesis of Ag nanoparticles using 1-(2-hydroxyethyl)-3-methylimidazolium tetrafluoroborate ([HEMIm][BF_4], ILa) and 1-(2'-hydroxyethyl)-2-methyl-3-dodecylimidazolium chloride ([C_{12}HEMIm][Cl], ILb) without any reductants, and show the preliminary findings of antimicrobial properties of the resulting Ag nanoparticles.

EXPERIMENTAL SECTION

1. Synthesis of Ionic Liquids

All the materials were used without additional further treatment. The route for the synthesis is illustrated in Scheme 1 and Scheme 2. Scheme 1 and Scheme 3 are described the structure of ionic liquids. The ionic liquids are synthesized in the literature [8-10]. To analyze ^{1}H NMR mass spectra, a Bruker DMX 600 MHz NMR spectrometer, and to record FAB mass spectra, a FAB mass JMS-HX110A were used. The content of residual halide ion was detected with the aid of ion chromatography (System: Bio-LC DX-300 (Dionex, Sunny-vale, CA, USA), Detector: Suppressed Conductivity (PED2), Column: ICSep AN300 with ICSep ANSC guard).

1-1. 1-(2-Hydroxyethyl)-3-methylimidazolium Chloride [HEMIm][Cl]

1-Methyl-imidazole (0.14 mol) reacted with an excess of hydroxyethyl chloride (2-chloroethanol, 0.2 mol) in a round-bottomed flask in a nitrogen atmosphere (70 °C, 48 hr), by using 200 mL of ace tonitrile as the solvent, thereby producing the 1-(2-hydroxyethyl)-3-methyl imidazolium halide ([HEMIm][Cl]). This molten salt of

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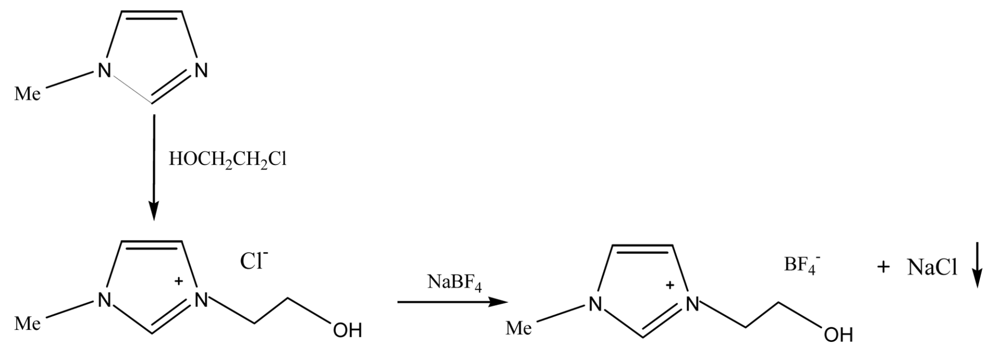
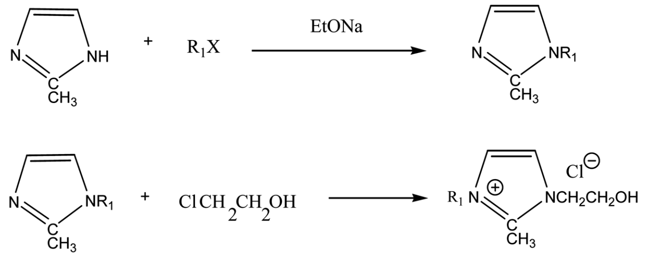
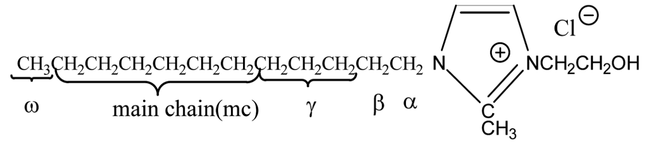


Scheme 1. Synthesis of [HEMIm][BF$_4$].



Scheme 2. Synthesis of [C$_{12}$HEMIm][Cl]; R$_1$=C$_{12}$H$_{25}$.



Scheme 3. Structural formula of [C$_{12}$HEMIm][Cl].

white crystalline solids was obtained by recrystallization under acetonitrile solvent in a freezer at −40 °C (yield 89%).

1-2. 1-(2-Hydroxyethyl)-3-methylimidazolium Tetrafluoroborate [HEMIm][BF$_4$]

The 1-(2-hydroxyethyl)-3-methyl imidazolium chloride was reacted with an equimolar amount of sodium tetrafloroborate in an acetone (25 °C, 24 hr), and the ionic liquid [HEMIm][BF$_4$] was formed. The sodium chloride was removed by filter paper and any residual sodium chloride in this IL was removed by low temperature filtration using celite. The organic liquid phase obtained from the filtration method was tested for the residual chloride salt through a concentrated AgNO$_3$ solution, and some precipitation of AgCl was confirmed with the naked eye. The ^{1}H-NMR(DMSO, δ/ppm, relative to TMS) spectrum consists of the following peaks: 3.72 (q, 2H), 3.88 (s, 3H), 4.21 (t, 2H), 5.29 (t, 1H), 7.71 (d, 1H), 7.75 (d, 1H), 9.18 (s, 1H). FAB mass showed m/z=127.17 [HEMIm]$^+$. The content of chloride anion was 24 ppm.

2. MIC Measurements

The antibacterial and antifungal activities of HIL-stabilized Ag nanoparticles were evaluated through measurements of minimal inhibitory concentrations (MIC) expressed in μg/ml. The MIC determination tests were carried out by an automatic serial dilution method in LB broth (microtitre). Bacterial and fungal inocula were prepared by diluting an overnight broth culture to yield an equivalent of approximately 10^6 cell mL^{-1}. Six microorganisms were used: *Bacillus subtilus* KCTC1914; *Staphylococcus aureus* 209 KCTC1916; and a few antibiotic resistant *Staphylococcus aureus* R209 KCTC1928 were chosen as test strains among gram-positive bacteria; *Escherichia coli* KCTC1924 and *Salmonella typhimurium* KCTC1926 were chosen from among gram-negative bacteria; and *Candida albicans* KCTC1940 was chosen as the representative fungus. Details of MIC test of Ag nanoparticles are described in Table 1.

Table 1. The MIC test of Ag nanoparticles stabilized by [HEMIm] [BF$_4$]

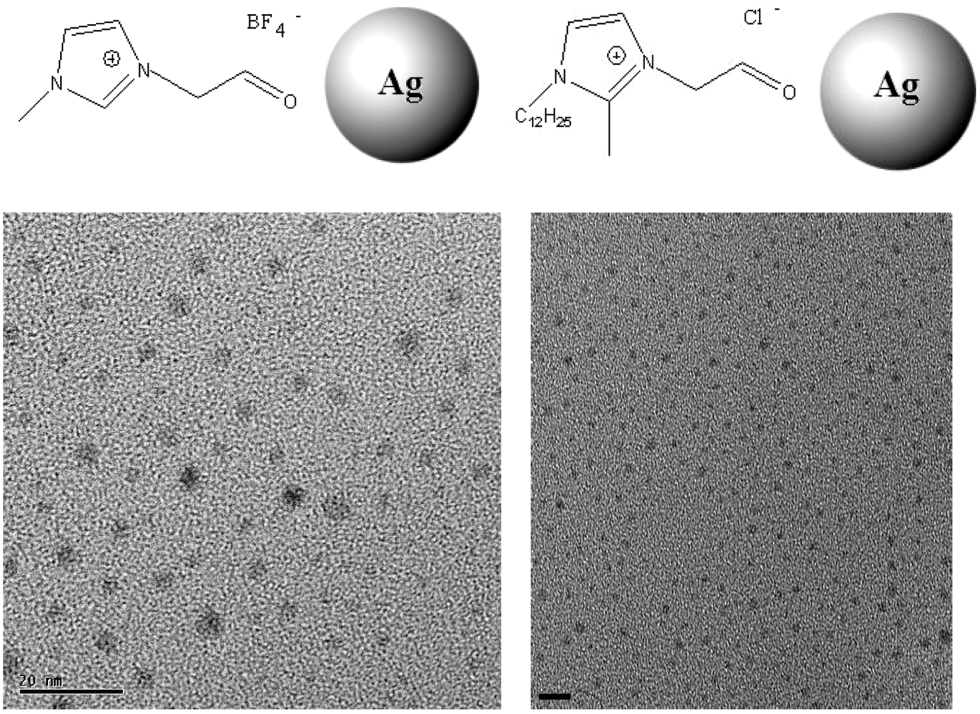
Compuond	MIC (μg/ml)					
	Tested organisms (bacteria and fungus)					
	EC[c]	ST[d]	SA[e]	SAR[f]	BS[g]	CA[h]
Ag nanoparticles	8	8	16	8	4	31
BAC[a]			8	8	8	
CPC[b]			8	8	8	

[a]BAC: benzalconium chloride. [b]CPC: cetylpyridinium chloride. [c]EC: escherichia coli KCTC1924. [d]ST: salmonella typhimurium KCTC 1926. [e]SA: staphylococcus aureus 209 KCTC1916. [f]SAR: staphylococcus aureus R209 KCTC1928. [g]BS: bacillus subtilus KCTC 1914. [h]CA: candida albicans KCTC1940.

RESULTS AND DISCUSSION

HILs can be considered to be the potential stabilizers for the following reasons. First, the synthesis and purification processes of HILs are much simpler than those of TFILs. Second, a reducing agent would not be needed because HILs simultaneously serve as the reductants and also as protective agents. Third, unlike in conventional solvents, the solvation and stabilization of metal ionic species in ILs facilitate both water and organic phase synthesis of nanoparticles. ILa and ILb were synthesized according to the standard method [4,8,11].

The one-phase preparation of Ag nanoparticles in water using ILa was straightforward, and purification of the nanoparticles was carried out in ambient conditions. 0.1 mmol of ILa in 10 ml of water was added dropwise to a solution of 0.1 mmol of silver nitrate (AgNO$_3$) aqueous solution in a round-bottomed flask under a nitrogen atmosphere with vigorous stirring at 70 °C. The IL-protected Ag nanoparticles (IL-Ag) were purified by removing unbounded HIL with



(a) (b)

Fig. 1. TEM images of HIL-stabilized Ag nanoparticles. Stabilizers: (a) ILa-Ag, (b) ILb-Ag, bar length: (a) 20 nm, (b) 10 nm.

ethanol, and were then centrifuged.

Fig. 1 displays a representative selection of the nearly spherical nanoparticles and indicates the particle size; the average diameter (Av) was 4.1 and 2.2 nm and the standard deviation (SD) representing the size distribution was 0.5 and 0.2 nm for ILa and ILb, respectively. The overall analysis shows a small and uniform IL-Ag. As an ion compound, HIL enables the easy solvation and stabilization of a metal salt in the water phase because the interaction between the IL and metal salt overcome the interaction between the water and metal salt. HIL functions as a reducing agent that reduces the Ag ion to Ag^0 and also acts as a stabilizer in forming Ag nanoparticles. It is thought that the $-CH_2OH-$ group in HIL is converted to the -CHO- group to reduce Ag ions, and simultaneously protect Ag nanoparticles according to the reaction Eq. (1) [12,13].

$$2Ag^{+}+[R\text{-}Im\text{-}CH_2CH_2OH]X \rightarrow 2Ag^{0}+[R\text{-}Im\text{-}CH_2CHO]X+2H^{+} \quad (1)$$

The formation of the aldehyde group in the suggested mechanism was confirmed by the color change based on a Purpald test [14,15]. Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) is known to specifically react with aldehydes in a 1 N NaOH solution, yielding intense purple derivatives in the solution. The IL-Ag in the Purpald solution reacted and turned purple after a 1 min reaction with the Purpald solution under N_2 followed by a 1 min air oxidation period. In contrast, there was no color change for any of the HIL.

One outstanding finding is that the size and distribution of nanoparticles strongly depend on the length of the side chain of cation. In terms of alkyl chain length, Fig. 1 shows that both the average size and standard deviation become smaller in the order of ILb> ILa, which suggests that the slow growth rate of Ag nanoparticles by $[C_{12}HEMIm][Cl]$ results in small and mono-dispersed Ag nanoparticles. These size differences might be due to the steric hindrance of the longer alkyl side chain.

Studies on the antimicrobial activities of pure ILs as one of the interesting applications have been reported [8,11]. Here a preliminary investigation on antibacterial and antifungal activities of ILa-Ag was performed through measurements of the minimal inhibitory concentrations (MIC) expressed in $\mu g\ mL^{-1}$, based on our previous study [8]. However, the antimicrobial activities of ILb-Ag were not carried out because ILb may be one of the surfactants which have antimicrobial potential. The MIC determination tests were performed by using the automatic serial dilution method in LB broth (microtitre). Bacterial and fungal inocula were prepared by diluting an overnight broth culture to obtain the equivalent of approximately 10^6 cell mL^{-1}. The MIC values of each Ag nanoparticle are shown in Table 1. ILa-Ag showed broader and higher bactericidal and fungicidal activity than that in the surfactants based on the imidazolium and pyrrolidinonium salts that were investigated in our previous work [8]. It is believed that the extremely large nano-scale surface area will allow for effective contact with the given microorganisms, leading to better MIC test results. We also compared the activity of IL-Ag with commercially produced and widely used quaternary ammonium substances (BAC and CPC). Details of MIC test of Ag nanoparticles are described in Table 1. ILa-Ag shows much broader antimicrobial activity spectra against fungus, Gram (+), and Gram (–) microbes compared to those of BAC and CPC. MIC tests were carried out for the [HEMIm][BF4] itself in order to confirm that the antimicrobial activity can be solely attributed to the Ag nanoparticles. [HEMIm][BF4] showed no activity against any of the test organisms. This shows that only the silver nanoparticles capped by the cation are responsible for the antimicrobial effect.

CONCLUSION

In summary, we have successfully prepared Ag nanoparticles in the water-phase via reduction by using a protective IL without adding any reductants. A new water-phase synthesis of silver nanoparticles using 1-(2-hydroxyethyl)-3-methylimidazolium tetrafluoroborate ([HEMIm][BF_4]) and 1-(2'-hydroxyethyl)-2-methyl-3-dodecylimidazolium chloride ([C_{12}HEMIm][Cl]) was described. The suggested mechanism of converting alcohol to aldehyde in the IL was supported by the Purpald test. We also found that the size of IL-Ag could be tuned by varying the side chain length of the cation. This method employs HIL as a stabilizer as well as a reductant, and is an efficient and effective strategy for preparing metal nanomaterials. Furthermore, though the MIC tests are preliminary, the IL-Ag is proven to act as a potential agent with both antibacterial and antifungal activity.

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