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Antimicrobial activity and cytotoxicity of piperazinium- and guanidinium-based ionic liquids

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HIGHLIGHTS

- Twelve piperazinium- and guanidinium-based ionic liquids were synthesized and characterized.
- Antimicrobial activities of the ionic liquids against *E. coli* and *S. aureus* were investigated.
- Cytotoxicity on the rat C6 glioma cells (C6) and human embryonic kidney cells (HEK-293) were evaluated.
- The ionic liquids with the $[BF_4]^-$ anion and with benzene ring on cation exhibit relatively high toxicity.

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ABSTRACT

Twelve piperazinium- and guanidinium-based ionic liquids (ILs) were synthesized, and characterized by ^{1}H nuclear magnetic resonance (NMR), thermal gravimetric analyzer (TGA) and differential scanning calorimetry (DSC). The antimicrobial activity and cytotoxicity have been investigated to provide the information whether the newly synthesized ILs are toxic or not. The antimicrobial effects of these ILs on gram negative and gram positive bacteria are evaluated on the basis of the minimum inhibitory concentration (MIC) measurements. The membrane damages of bacteria in the presence of ILs are observed by scanning electron microscopy (SEM). The cytotoxicity data of the ILs on HEK-293 and C6 cells are obtained by MTT cell viability assay. The disruption of cell cycle is analyzed by the flow cytometry. The results show that most of the ILs exhibit low toxicity, and the ILs with tetrafluoroborate anion and with benzene ring on cation are the species with relatively high toxicity among the studied ILs. The fundamental data and results can provide some useful information for the further studies and applications of the ILs.

1. Introduction

Ionic liquids (ILs) have generally been considered as green solvents for their negligible vapor pressure and good thermal stability. They are entirely ionic in nature, and have relatively high polarity. The melting point temperatures of ILs are usually lower than 100 °C, and the ILs can be at liquid state over a large temperature range [1,2]. ILs can be designed for a particular function from the choice of anion or cation to change the physical and chemical properties [3,4]. Abundant researches have indicated that ILs can be utilized for a wide variety of applications, such as organic synthesis [5,6], catalysis [7,8], extraction process [9], and electrochemistry [10].

For the requirements of low toxicity and environmental safety of chemicals [11–13], it is necessary to carry out studies on the toxicity of any novel ILs. The toxicology properties are of critical importance for the ongoing utilization of ILs. During the past years, some of the toxicological studies on ILs have been introduced against different targets [14]. They are mainly focused on the effects on growth inhibition of bacteria [15], fungi [16], eukaryotic cells [17–19], unicellular algae [20,21], and aquatic or terrestrial plants [3,22]. The investigated ILs involve those composed of imidazolium [22], morpholinium [4] or quinolinium [23,24] cations with bulky chain substituents, and various anions including inorganic (chloride, bromide, nitrate, tetrafluoroborate, hexafluorophosphate, hydrogen sulphate) and organic (acetate, methoxyacetate, dodecylsulfate, lactate, salicylate, etc.) ones. A general trend is found that the toxicity of an IL can be related to the lipophilicity [4], and the IL with a longer alkyl substituent is usually more toxic than that with a shorter alkyl chain [25]. The toxicity can be remarkably reduced

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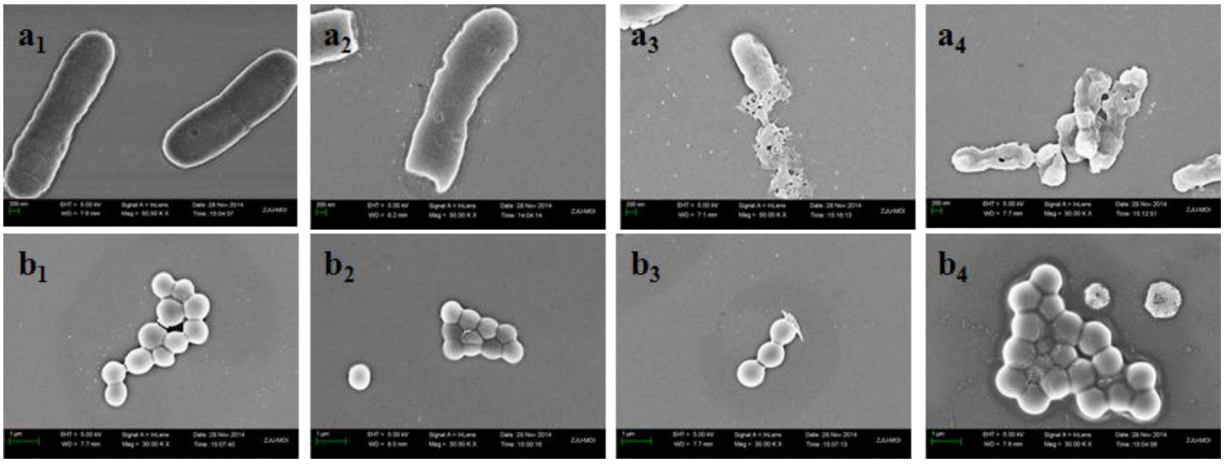

Fig. 1. SEM images of the bacteria treated with $[C_2pi][BF_4]$ for 12 h. a_1, *E. coli* control, untreated; a_2, *E. coli* treated with 0.1 mg mL^{-1} IL; a_3, *E. coli* treated with 1.0 mg mL^{-1} IL; a_4, *E. coli* treated with 2.0 mg mL^{-1} IL; b_1, *S. aureus* control, untreated; b_2, *S. aureus* treated with 0.1 mg mL^{-1} IL; b_3, *S. aureus* treated with 1.0 mg mL^{-1} IL; b_4, *S. aureus* treated with 2.0 mg mL^{-1} IL.

when polar functional groups are presented instead of non-polar functional groups on the cations [26]. In most cases, the cation containing a long side chain shows more significant contribution to the toxicity than the anion does. Several fluorinated hydrophobic anions, however, show extremely high toxicity [27,28]. Stolte et al. [3]. reported the influences on the toxicity from different head groups, functionalized side chains, and anions of ILs. Pernak et al. [4]. discussed the cytotoxicity and biodegradability of morpholinium-based ILs. It was found that the cytotoxicity and the antimicrobial activity of these morpholinium ILs were much lower than those of the commonly applied imidazolium- and pyridinium-based ILs.

In our laboratory, a series of piperazinium-based ILs have been recently synthesized [29–31], and some of them have been utilized for fuel desulfurization and have shown satisfactory performance. The guanidinium-based ILs have already been used for extraction of aromatics from hydrocarbon fuels [30], and absorption of SO_2 [32–36]. These applied ILs compose a new family of ILs with simple structures, and the potential toxicity of them is expected to be smaller than that of the classical ILs such as imidazolium-based ones. The aim of the present work is to evaluate the toxicological impacts of piperazinium- and guanidinium-based ILs with different anions, such as tetrafluoroborate, lactate, iodide and ethyl sulfate. The antimicrobial activities of these ILs against *Escherichia coli* and *Staphylococcus aureus* are determined by using the MIC method, and the cytotoxicity data of ILs on HEK-293 and C6 cells are investigated by MTT cell viability assay and cell cycle analyses.

2. Experimental

2.1. Syntheses of ILs

1-Methylpiperazinium (mass fraction >99.0%, Aldrich), 1,4-dimethylpiperazinium (>98.0%, Aldrich), 1-ethylpiperazinium (>98.0%, Aldrich), 1-phenylpiperazinium (>98.0%, Aldrich), 1,1,3,3-tetramethylguanidine (>99.0%, Aldrich), tetrafluoroboric acid (∼50.0% aq., Sigma–Aldrich), lactic acid (>90.0%, Fluka), and iodoethane (>99.0%, Sigma–Aldrich) were used without purification. The piperazinium- and guanidinium-based ILs were synthesized with the following procedures (Scheme 1).

Strategy I: piperazinium-based ILs were synthesized by the dropwise addition of tetrafluoroboric acid, lactic acid or iodoalkane (0.1 mol) dissolved in ethanol or ethyl acetate (50 mL) into the solution of alkylpiperazine (0.1 mol) in ethanol or ethyl acetate (50 mL) in an ice–water bath for 12 h. The solvent was then removed by evaporation, and the products were washed with ethyl acetate and dried under vacuum (<10 Pa) at 50 °C for 24 h.

Strategy II: 2-ethyl-1,1,3,3-tetramethylguanidinium ethyl sulfate ($[C_2{}^2(C_1)_2(C_1)_2{}^3gu][C_2OSO_3]$): the mixture of diethyl sulfate (DES, 0.05 mol) in ethyl acetate (50 mL) was added dropwise into 1,1,3,3-tetramethylguanidine (0.05 mol) in ethyl actate (50 mL). The reaction lasted for 24 h in an ice–water bath with a magnetic stirring. The lower phase was washed with ethyl acetate and the volatile components were removed by evaporation. The products were dried and stored in a dry nitrogen atmosphere.

2,2-Diethyl-1,1,3,3-tetramethylguanidinium ethyl sulfate ($[(C_2)_2{}^2(C_1)_2(C_1)_2{}^3gu][C_2OSO_3]$): 1,1,3,3-tetramethylguanidine (0.05 mol) in dichloromethane (50 mL) was dropwise added into diethyl sulfate (0.10 mol) in dichloromethane (100 mL), whereafter solid NaOH (∼0.06 mol) was added into the mixture. The reaction lasted for 48 h in an ice–water bath under the protection of nitrogen gas. Then, the mixture was filtered. After removal of solvent, the filtrate was washed with ethyl acetate. The products were dried and stored in a dry nitrogen atmosphere.

2-Ethyl-1,1,3,3-tetramethylguanidinium iodide ($[C_2{}^2(C_1)_2(C_1)_2{}^3gu][I]$): 1,1,3,3-tetramethylguanidine (0.10 mol) and iodoethane (0.10 mol) were reacted in ethyl acetate for 24 h in an ice–water bath. The precipitate was recrystallized and washed with ethyl acetate. Thereafter, $[C_2{}^2(C_1)_2(C_1)_2{}^3gu][I]$ was obtained as colorless crystals.

2,2-Diethyl-1,1,3,3-tetramethylguanidinium iodide ($[(C_2)_2{}^2(C_1)_2(C_1)_2{}^3gu][I]$): 1,1,3,3-tetramethylguanidine (0.1 mol), solid NaOH (c.a. 0.11 mol) and iodoethane (0.20 mol) were added together into dichloromethane. The reaction lasted for 48 h in an ice–water bath. After filtration, the crude product was further recrystallized and washed with ethyl acetate. $[(C_2)_2{}^2(C_1)_2(C_1)_2{}^3gu][I]$ was obtained as colorless crystals.

2.2. Characterizations of ILs

The ILs were characterized by ^1H nuclear magnetic resonance (NMR) performed on a Bruker AVANCE 500 MHz NMR spectrometer, using $CDCl_3$, D_2O or CD_3OD as the solvent.

The thermal stability of the ILs was characterized using a thermal gravimetric analyzer (TGA, TA Instruments, Q50) under a nitrogen atmosphere. Each IL sample about 5.0 mg (Mettler Toledo, XS105) was loaded into a ceramic crucible and heated at the heating rate of 10 °C min^{-1} from the room temperature to 550 °C. The