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Short Communication

Assessment of cytotoxicity and toxicity for phosphonium-based deep eutectic solvents

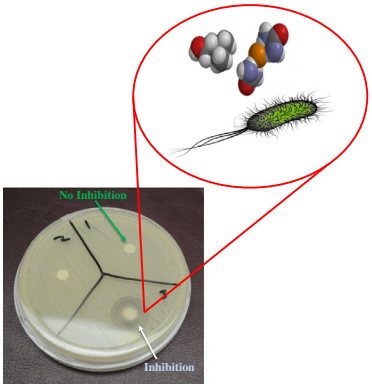
Maan Hayyan [a,b,*], Mohd Ali Hashim [b], Mohammed A. Al-Saadi [b], Adeeb Hayyan [b], Inas M. AlNashef [c], Mohamed E.S. Mirghani [d]

[a] Department of Civil Engineering, Faculty of Engineering, University of Malaya, Kuala Lumpur 50603, Malaysia
[b] University of Malaya Centre for Ionic Liquids (UMCiL), Department of Chemical Engineering, University of Malaya, Kuala Lumpur 50603, Malaysia
[c] Chemical Engineering Department, King Saud University, Riyadh 11421, Saudi Arabia
[d] Department of Biotechnology Engineering, International Islamic University Malaysia, Kuala Lumpur 50728, Malaysia

HIGHLIGHTS

- This is the first time cytotoxicity and toxicity of phosphonium-based DESs were studied.
- Cytotoxicity of tested DESs was much higher than their individual components.
- This indicates the synergistic effect after DES forming.
- There was a toxic effect for DESs on studied bacteria.
- This indicates the potential application of DESs as anti-bacterial agents.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, the cytotoxicity and toxicity of phosphonium-based deep eutectic solvents (DESs) with three hydrogen bond donors, namely glycerine, ethylene glycol, and triethylene glycol were investigated. The cytotoxicity effect was tested using brine shrimp (*Artemia salina*). The toxicity was investigated using the two Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. The cytotoxicity of tested DESs was much higher than that of their individual components, indicating their toxicological behavior was different. It was also found that there was toxic effect on the studied bacteria, indicating their potential application as anti-bacterial agents. To the best of our knowledge, this is the first time the cytotoxicity and toxicity of phosphonium-based DESs were studied.

1. Introduction

Deep eutectic solvents (DESs) as low cost alternatives to conventional ionic liquids (ILs) have currently been a subject of extensive research. The physicochemical properties of DESs, such as density, viscosity, refractive index, conductivity, surface tension and chemical inertness are very close to those of common ILs (Zhang et al., 2012). DESs possess numerous advantages in com-

* Corresponding author. Address: Department of Civil Engineering, Faculty of Engineering, University of Malaya, Kuala Lumpur 50603, Malaysia. Tel./fax: +60 3 7967 5311.
E-mail address: maan_hayyan@yahoo.com (M. Hayyan).

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parison to ILs in terms of ease of synthesis and low production cost (Jhong et al., 2009; Hayyan et al., 2012; Singh et al., 2012).

DESs are utilized in many applications such as biological catalysis (Durand et al., 2012), lubrication (Shi et al., 2013), electrochemical processes (Abbott et al., 2007), synthesis of solar cells (Steichen et al., 2011), production and purification of biodiesel (Hayyan et al., 2010, 2013a), separation of aliphatic and aromatics (Kareem et al., 2012), and many other potential applications.

DES is a mixture of two or more compounds which has a melting point lower than that of either of its components (Hayyan et al., 2010). This new mixture can generally be identified and characterized by a lower freezing point than that of the individual constituents. The charge delocalization occurring through hydrogen bonding between the halide anion and the hydrogen bond donor moiety is responsible for the decrease in the freezing point of the mixture relative to the melting points of the individual components (Carriazo et al., 2012).

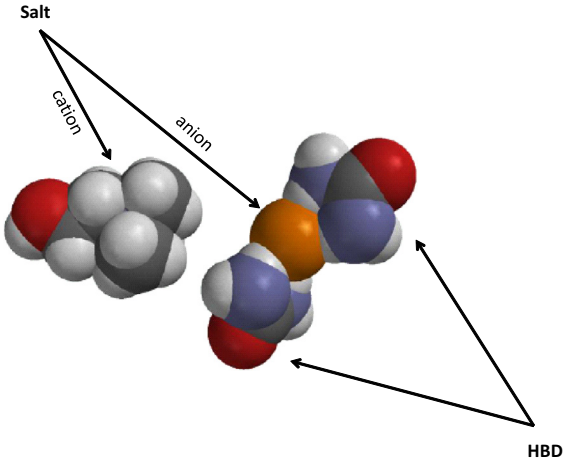
Abbot's group reported the synthesis of DESs based on mixtures of quaternary ammonium salts such as chlorine chloride (ChCl) with hydrogen bond donors (HBDs) such as amines and carboxylic acids, Scheme 1. The deep eutectic phenomenon was first described for a mixture of ChCl and urea with a mole ratio of 1:2 (Abbott et al., 2004). The freezing point of this eutectic is 12 °C, which is considerably lower than that of ChCl and urea (melting point of ChCl and urea are 302 and 133 °C, respectively). This significant depression of the freezing point stems from an interaction between the halide anion of the salt and the HBD component (Hayyan et al., 2010; Zhang et al., 2012).

In 2010, a new generation of DESs based on phosphonium salts was successfully synthesized and introduced (Kareem et al., 2010). The melting point of many of these DESs are lower than 100 deg C. To date, the toxicity of these DESs has not been reported but numerous reports (Abbott et al., 2004; Jhong et al., 2009; Hayyan et al., 2012; Singh et al., 2012; Wu et al., 2012) claim that, in general, DESs are non-toxic, eco-friendly, biodegradable and benign solvents. These information were based on the properties of individual components of the studied DESs. Recently, we have shown that ammonium-based DESs have cytotoxicity higher than their individual components (Hayyan et al., 2013b). A number of reasons were suspected to cause this cytotoxicity such as hydrogen bonding between the HBD and the anion of the salt that forms the DES, the lack of oxygen, or the difficulty in the brine shrimp movement due to the high viscosity of DES (Hayyan et al., 2013b).

Although DESs demonstrated excellent performance in a wide range of applications their use in industry is very limited due to the lack of data concerning their corrosivity, biodegradability and toxicity. Furthermore, since none of these DESs are not yet registered, their general use as solvents may be restricted. Therefore, the cytotoxicity and toxicity of DESs are fundamental aspects that must be addressed before their applications.

The toxicity of conventional phosphonium-based ILs was investigated by Ventura et al. (2012). The toxicity results obtained for the phosphonium family suggested that the long alkyl chains promote higher toxic effects towards the studied bacterium. Furthermore, the phosphonium-based ILs are more toxic than the analog imidazolium-based ILs.

In this work, the cytotoxicity of selected phosphonium-based DESs towards brine shrimp (*Artemia salina*) was investigated. Furthermore, the toxicity of these DESs using two Gram positive bacteria, i.e. *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram negative bacteria, i.e. *Escherichia coli* and *Pseudomonas aeruginosa*, has been investigated. The DESs are based on methyltriphenylphosphonium bromide (MTPB) combined with three HBDs including glycerine (Gl), ethylene glycol (EG) and triethylene glycol (TEG), Table 1 and Scheme 2.



Scheme 1. Structure of a DES (Abbott et al., 2004).

2. Experimental methodology

2.1. Synthesis of DES

To prepare the DESs used in this work MTPB (Merck 99%) was dried under vacuum and mixed with the HBD (Merck) in mole ratio 1:3 MTPB to HBD. The mixture was stirred at 300 rpm at a temperature of 80 °C until a homogenous transparent liquid was formed.

2.2. Antibacterial assay

The Gram positive and Gram negative bacteria were pre-cultured in by transferring loopful of cells into already autoclaved LB broth and allowed to incubate at 37 °C for 24 h. The standard cells were spread on the plates by using sterile cotton bud.

2.3. Filter paper diffusion assay

Sterile 6 mm filter paper (Whatman No. 1) was soaked with solutions and allowed to equilibrate before placing on the seeded plates and allowed to stand before incubation. The plates were then incubated at 37 °C for 24 h before the diameters of zones of inhibition were then measured. These were repeated in three replicates for each of the tested organism.

2.4. Brine shrimp assay

2.4.1. Preparation of simulated seawater

Sea-salt (non-iodized NaCl) was weighed accurately (38 g) and dissolved in 1 L of sterilized distilled water. The solution was stirred for total salt dissolution.

2.4.2. Hatching of brine shrimp eggs

A. salina leach (brine shrimp) was used as the test organism. Simulated seawater was added into a small tank and the shrimp eggs (1.5 g L^{-1}) were added to one side of the tank. The shrimps hatched after 2 d and matured as nauplii (larvae). The tank was occasionally stirred for oxygen penetration during the hatching period. These nauplii were taken for this bioassay. About 10 cells were transferred from the pond into the solution. Cells were observed under light for lethality.