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Identifying the component responsible for antagonism within ionic liquid mixtures using the up-to-down procedure integrated with a uniform design ray method

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ABSTRACT

Various chemicals in the environment always exist as mixtures. Toxicity interaction within mixtures may pose potential hazards and risks to the environmental safety and human health. Recent studies showed that toxicity interaction by ionic liquid (IL) mixtures can be related to a certain component. To identify the component, we developed a novel procedure integrating an up-to-down process with the uniform design-based ray method (UDUD) and applied it into an IL mixture system of four 1-butyl-3-methylimidazolium ILs (simply [bmim]X) where X=Cl^-, Br^-, $CH_3OSO_3^-$ and $CH_3(CH_2)_7OSO_3^-$. It was shown that two mixture rays in the quaternary system exhibited significant antagonistic interaction. In this paper, the UDUD was first employed to design four ternary mixture systems. The microplate toxicity analysis was used to determine the toxicities of various mixtures to a freshwater photobacterium *Vibrio qinghaiensis* sp.-Q67. The concentration addition was taken as an additive reference to assess the toxicity interactions taking place in mixtures. The results revealed that some ternary mixture rays including [bmim]$CH_3(CH_2)_7OSO_3$ display antagonism while the ternary rays without [bmim]$CH_3(CH_2)_7OSO_3$ exhibit additivity. On these grounds, we again designed all binary mixtures containing [bmim]$CH_3(CH_2)_7OSO_3$, determined their toxicities and assessed toxicity interaction. The results showed that three binary mixture systems produce antagonism. Thus, it may be concluded that [bmim]$CH_3(CH_2)_7OSO_3$ is indeed a key component inducing mixture antagonism.

1. Introduction

In a real environment, various chemicals always exist as mixtures. The effect of a chemical mixture has different features from the components alone and cannot be always predicted by the concentration–response information of pure components in the mixture (Liu et al., 2012). If the behavior of environmental contaminants in a mixture is not consistent to that predicted by an additive reference model, the mixture may induce higher or lower toxicity (Altenburger et al., 2003; Hernández et al., 2013). The former is called synergism and the latter antagonism. The synergism or antagonism (toxicity interaction) of mixtures has potential hazards and risks to the environment and human health (Goldoni and Johansson, 2007; Groten et al., 2001). Therefore, screening out the mixtures exhibiting synergism or antagonism is very important. Recent research showed that toxicity interaction (synergism or antagonism) may be attributed to the existence of a certain component in mixtures (Zhang et al., 2012a). However, how to identify and determine the specific component in a multi-component mixture is not an easy work and needs to be systematically studied (Jonker et al., 2005).

A rational experimental design is helpful to detect the toxicity interaction of mixture components. One of traditional designs is the factorial design. However, the number of the mixture experiments in the factorial design is exponentially increased with the number of compounds in the mixture (Meadows et al., 2002). Although the fixed ratio ray design (FRRD) was proposed to largely reduce the amount of experimental effort when interest can be restricted to a specific ray (Meadows et al., 2002; Moser et al., 2005), the mixtures designed by FRRD merely represent a direction in the concentration space constructed by the mixture components and cannot characterize the whole mixtures (Zhang et al., 2010). More and more facts showed that the toxicity interaction may depend on not only the

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concentration level of the mixtures but also the concentration ratios of a components in the mixtures (Jonker et al., 2005); (Zhang et al., 2012b). So, it is necessary to design and consider many mixture rays with various concentration ratios. The UD-Ray experimental design combining the uniform design with FRRD was proved to be suitable to study the combined toxicity of a series of mixtures with a defined composition (Liu et al., 2012; Zhang et al., 2010, 2011).

Ionic liquids (ILs) have gained considerable attention as 'green' media for a wide range of synthetic and analytical processes due to several attractive properties such as high thermal stability, non-flammability and negligible vapor pressure which reduces their atmospheric release (Fernandez et al., 2011; Quijano et al., 2010; Xia et al., 2012; Yang et al., 2012). However, the green credentials of ILs are being challenged due to the increasing evidence of their toxicity (Samori, 2011; Thi et al., 2010; Ventura et al., 2012; Viboud et al., 2012). In addition, some IL mixtures, consisting of many ILs or IL and the other chemicals such as pesticides or metals, can produce synergism or antagonism (Dou et al., 2011; Matzke et al., 2008; Zhang et al., 2009). Our recent studies (Zhang et al., 2011) found that some quaternary IL mixtures exhibit significant antagonism (see Fig. S1 in Supplementary material). How to analyze and identify the key component inducing the antagonism in the mixtures forces us to continue the work.

The main aim of this paper is to provide a novel procedure for the identification of the key component responsible for the antagonism in the quaternary mixtures. The procedure integrated the up-to-down method with the UD-Ray design (so called the UDUD). In the UDUD procedure, the toxicity interaction of the representative mixtures in all ternary mixture systems of four ILs was first identified. Based on the toxicity interaction results, the interaction in the relative binary mixtures was furthermore examined. The representative mixtures were designed by the UD-Ray where five mixture rays were constructed for each mixture system and twelve mixtures were assigned for each mixture ray. The toxicities of all mixtures to the freshwater photobacterium *Vibrio qinghaiensis* sp.-Q67 (*V. qinghaiensis*) were determined by using the microplate toxicity analysis (MTA) procedure (Liu et al., 2009, 2012; Zhang et al., 2008). The toxicity interaction was identified by selecting the concentration addition (CA) as an additive reference model (Liu et al., 2013; Zhang et al., 2011).

2. Materials and methods

2.1. Test materials

Three ionic liquids (ILs), 1-butyl-3-methylimidazolium chloride ([bmim]Cl, CAS RN 79917-90-1), 1-butyl-3-methylimidazolium bromine ([bmim]Br, 85100-77-2) and 1-butyl-3-methylimidazolium methylsulfate ([bmim]CH_3OSO_3 or [bmim]CH_3SO_4, 401788-98-5), were purchased from ACROS, and another IL, 1-butyl-3-methylimidazolium octyl sulfate ([bmim]$CH_3(CH_2)_7OSO_3$ or [bmim]$C_7H_{17}SO_4$, 445473-58-5) was purchased from Strem Chemical (see Table S1 in Supplementary material). All tested ILs with the purity of >97 percent are miscible with water. The stock solution was prepared by dissolving ILs in milli-Q water and stored in dark at 4 °C. The reason why the ILs are selected is that some of their quaternary mixtures have significant antagonistic action rather than additive action in our previous work (Zhang et al., 2011).

2.2. Mixture design

To identify the key component inducing the mixture interaction such as antagonism, we adopted the up-to-down procedure to design the mixture systems (Fig. 1). In the up-to-down method, we first employed the uniform design to effectively describe the concentration composition for each mixture system and used the ray design to extend the concentration composition (point) as a ray (Liu et al., 2012). Then, the toxicity interaction (synergism or antagonism) in the quaternary mixture system is examined. If there is significant interaction, the interaction in the next ternary mixture system is then examined, and so on.

To validate the applicability of the UDUD method above, we examined all combinations of four ILs under study. These combinations include one quaternary mixture system (Q), four ternary systems (T1–T4) and six binary ones (B1–B6). The quaternary system Q contains all the four ILs, [bmim]Cl, [bmim]Br, [bmim]CH_3SO_4 and [bmim]$C_8H_{17}SO_4$. Four ternary systems contain three of four ILs: [bmim]Cl, [bmim]Br and [bmim]CH_3SO_4 for T1; [bmim]Br, [bmim]CH_3SO_4 and [bmim]$C_8H_{17}SO_4$ for T2; [bmim]Cl, [bmim]Br and [bmim]$C_8H_{17}SO_4$ for T3; [bmim]Cl, [bmim]CH_3SO_4 and [bmim]$C_8H_{17}SO_4$ for T4. Six binary systems contain two ILs: [bmim]Br and [bmim]CH_3SO_4 for B1; [bmim]Cl and [bmim]Br for B2; [bmim]Cl and [bmim]CH_3SO_4 for B3; [bmim]Cl and [bmim]$C_8H_{17}SO_4$ for B4; [bmim]Br and [bmim]$C_8H_{17}SO_4$ for B5; and [bmim]CH_3SO_4 and [bmim]$C_8H_{17}SO_4$ for B6. For each mixture system, five mixture rays (R1, R2, R3, R4, and R5) were designed by the UD-ray. For each mixture ray, twelve mixtures were assigned by the fixed concentration ratio procedure. So, there were twenty rays for the ternary mixtures and 30 for the binary mixture systems. The concentration ratios (p_is) of components, the ratio of the concentration of a component to the total concentration of the mixture, in various mixture rays are listed in Table 1.

2.3. Microplate toxicity analysis

The toxic effects (or percent inhibition) of IL mixtures to *V. qinghaiensis* were determined by the microplate toxicity analysis (MTA) developed in our previous

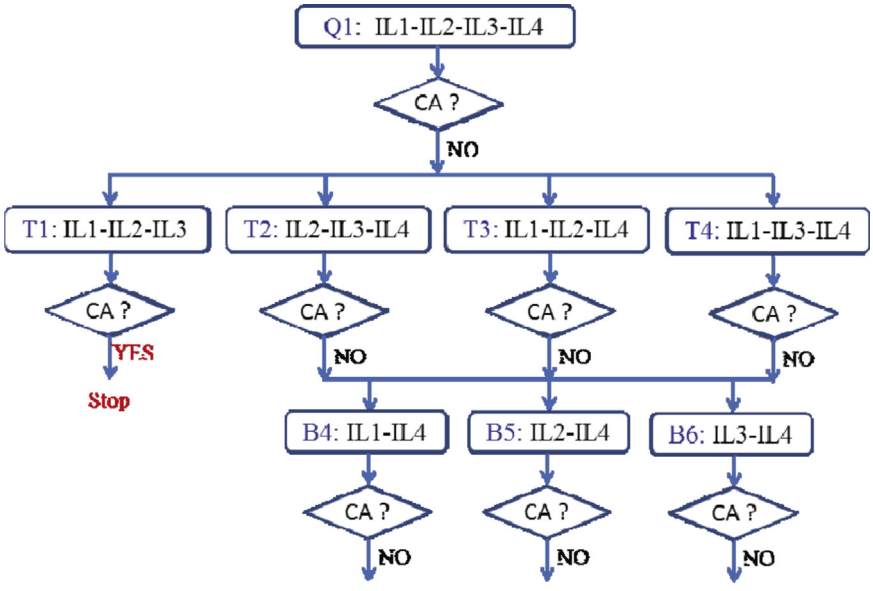

Fig. 1. Diagrammatic sketch of the up-to-down procedure identifying the key component where CA refers to the concentration addition and IL1, IL2, IL3 and IL4 refer to [bmim]Cl, [bmim]Br, [bmim]CH_3SO_4 and [bmim]$C_8H_{17}SO_4$, respectively.