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The antagonist and synergist potential of cholinium-based deep eutectic solvents

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ABSTRACT

The design of environmentally friendlier solvents has gained increasing relevance in the last decade. Deep eutectic solvents (DES) have recently emerged, with advantages like low-cost and putative lower environmental impact. However, information about DES toxicity is still scarce. This work aims to contribute to profiling the ecotoxicity of DES based on cholinium chloride ([Chol]Cl). Six DES were addressed, combining [Chol]Cl (as hydrogen bond acceptor – HBA) with ethylene glycol, glycerol, 1,2-propanediol, propionic acid, 1-propanol, and urea as hydrogen bond donors (HBD), in different molar ratios. The Microtox® Acute Toxicity Test, was used for assessing their toxicity towards the marine bacteria *Allivibrio fischeri* . Because the dissociation of DES in water is expected, analysis appraising the mixtures toxicity theory should be considered, which is a step forward in this field. This analysis suggested that [Chol]Cl and all HBD with the exception of propionic acid:[Chol]Cl 1:2 and 4:1 behave antagonistically, which is contrary to what has been suggested previously. The most extreme cases are Urea:[Chol]Cl and 1-Propanol:[Chol]Cl, with EC_{50} values higher than their starting materials dosed singly, configuring very promising and biocompatible alternative solvents. Toxicity was found to be dependent on DES composition, as well as on molar proportions of the starting materials.

1. Introduction

The concept of “Green Chemistry” was introduced in the early 1990's with the propose of designing and applying chemical products and processes in order to reduce, or preferentially eliminate, the use and generation of hazardous substances (Anastas and Warner, 1998). In this field, the design of environmentally friendlier solvents compared to their traditional counterparts has gained increased attention.

Deep eutectic solvents (DES) emerged in this context. They are simple to prepare and do not need purification, have low-cost production due to the low cost of starting materials, and have been showing good biocompatibility (Hayyan et al., 2013b; Jhong et al., 2009; Singh et al., 2012). These solvents were first developed combining urea with cholinium chloride (Abbott et al., 2003), but DES can be prepared through the mixing of two or three different components belonging to different chemical families (e.g., quaternary ammonium salts, amides, organic acids, polyalcohols), forming an eutectic mixture based on hydrogen bonding interactions between the components, with a melting point much lower than either of the individual components (Dai et al., 2013; Ruß and König, 2012; Zhang et al., 2012). The melting point depression of DES is hypothesized to be caused by charge delocalization due to the hydrogen bonding between the halide anion (hydrogen bond acceptor; HBA) and the hydrogen bond donor (HBD) (Abbott et al., 2003). However, recent studies using ab initio molecular dynamic simulations were developed to gain insights on the charge spreading in the liquid state and casted strong doubts on this hypothesis (Zahn et al., 2016).

DES can be used in several applications in areas such as synthesis, metal-catalysed organic reactions and biocatalysis, electrochemistry, nanomaterial's, extraction and purification processes and in the pharmaceutical and biomedical fields (Farias et al., 2017; Mbous et al., 2017a; Smith et al., 2014; Tang and Row, 2013; Zhang et al., 2012). However, the application of these solvents at an industrial scale requires the previous knowledge of their environmental impact and fate (e.g. biodegradation and ecotoxicity) (Radošević et al., 2015). Studies about DES toxicity are still scarce to fully understand their toxicological

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profile, and the overall assumption of the DES benign character is mostly based on the low toxicity of their precursors (Radošević et al., 2015). However, this assumption disregards putative interaction effects between the DES compounds, including synergic toxic effects (Hayyan et al., 2013b). The toxicity of cholinium chloride and phosphonium-based DES was assessed through organisms, namely crustaceans (Hayyan et al., 2013a, 2013b), bacteria (De Morais et al., 2015; Hayyan et al., 2013a, 2013b; Zhao et al., 2015; Wen et al., 2015), fungi (Cardellini et al., 2015; Juneidi et al., 2016), plants (Radošević et al., 2015; Wen et al., 2015), invertebrates (Huang et al., 2014; Wen et al., 2015), fish (Juneidi et al., 2016), mice (Hayyan et al., 2015), and several cell lines (Hayyan et al., 2016, 2015; Mbous et al., 2017b; Paiva et al., 2014). The ecotoxicological profile of the DES previously studied did not highlight any specific rule, being the toxic effects dependent on DES composition, concentration, and test model. This hampers the application of predictive models of DES ecotoxicity, thus requiring its specific characterization, before generally concluding on their benign character and their suitability for large-scale use.

The aim of this study is to evaluate the ecotoxicity of cholinium chloride-based DES, since this is one of the widespread precursors most used in their formation. Cholinium chloride or (2-hydroxyethyl)trimethylammonium chloride) abbreviated in this work as [Chol]Cl, is a quaternary ammonium salt member of vitamin B family and used in several metabolic pathways (Florindo et al., 2014). It is cheap, biodegradable and non-toxic, and it is approved as a nutritional additive for animals (FEEDAP, 2011). Six [Chol]Cl-based DES containing ethylene glycol, glycerol, 1,2-propanediol, propionic acid, 1-propanol, and urea as HBD, in different molar ratios, were screened for their environmental hazardous potential using the Microtox® Acute Toxicity Test. This sensitive and widely accepted test (Johnson, 2005) allowed us to gain specific insights on the role of the HBD and molar ratio between HBA and HBD in the overall toxicity of [Chol]Cl-based DES. Considering the dissociating nature of DES when standing in a significant amount of water (Dai et al., 2015; Passos et al., 2016), this work provides a basic analysis of their toxicity considering them as binary mixtures of two precursors. In this context, the toxicity of different DES analyzed as mixtures was compared to that of their precursors.

2. Material and methods

2.1. DES preparation

The following chemical compounds were used for DES preparation: [Chol]Cl (98% purity) was purchased from Acros Organic®, Geel, Belgium; ethylene glycol (99.5% purity) was purchased from Sigma Aldrich®, St. Louis, Missouri, EUA; 1,2-propanediol from Panreac®, Barcelona, Spain; propionic acid (99% purity) from Merck®, Darmstadt, Germany; glycerol (99,8% purity) was purchased from Fischer Chemical®, Hampton, New Hampshire, EUA; urea (99% purity) from Panreac®, Barcelona, Spain; and 1-propanol (99.5% purity) was acquired from Merck®, Darmstadt, Germany.

Each DES was prepared in the following molar ratios: 1:2, 1:1, 2:1 and 4:1 (HBD:HBA). Briefly, HBD and HBA were added gravimetrically to closed vials and heated with constant agitation. After the formation of a transparent liquid, the mixture was cooled down to room temperature to obtain each DES. Since the purpose of this study was the evaluation of the ecotoxicological character of DES, some of the stock eutectic mixtures were prepared by adding a known volume of water. The water content of the starting materials and of DES was determined by Karl Fischer titration and considered in the calculation of the toxicity (Table S1).

2.2. Microtox® Acute Toxicity Test

The Microtox® Toxicity Test (Microbics Corporation, 1992) was used to assess the toxicity of the prepared DES as well as the starting materials, through the inhibition of the luminescence of the marine bacteria *Allivibrio fischeri*. This test was performed using a range of diluted aqueous solutions (from 0% to 81.9%) of each stock solution. After 5, 15, and 30 min of exposure to the test dilutions, the light output of the luminescent bacteria was measured and compared with the light output of a blank control sample. In this work, the concentrations of each sample tested were not checked before the toxicity measurements. The reasoning for this option combines three essential features of the established test system. First, the exposure period is very short (30 min), which renders very unlikely any significant degradation; degradation of these compounds under the conditions of this specific assay was not evidenced so far. Then, under these conditions, [Chol]Cl and each HBD used in this work are completely soluble in water (Farias et al., 2018; Vieira et al., 2018), which renders the final solutions stable. Finally, the focus here was in the methodologies used for the analysis of DES toxicity rather than on establishing actual effect concentrations, although we estimate them for internal comparison purposes. These data were used to estimate the concentrations that promote 50%, 20% and 10% of luminescence inhibition (Effective Concentration, EC_{50}, EC_{20} and EC_{10}, respectively) and the corresponding 95% confidence intervals through a non-linear regression, using the least-squares method to fit the data to the logistic equation. These analyses were performed using STATISTICA, version 8.0 software (StatSoft, 2007). This test was applied to DES and starting materials described above.

2.3. Mixture toxicity assessment

In order to compare DES toxicity with the toxicity of corresponding starting materials, we assumed that each DES is a mixture composed by the two starting materials as all concentrations tested in the Microtox® assay comprise more than 18.3% of water (note that only in the highest concentration tested (81.9%) the amount of water is less than 50%) (see Table S1). A basic approach to primarily address mixtures toxicity is the application of the model of Concentration Addition (CA) for the joint action of chemicals (Berenbaum, 1985). CA assumes that mixture components act as dilutions of each other because they have a similar mode of action. DES are thought to act as membrane disruptors (Hayyan et al., 2015; Mbous et al., 2017b), thus we are assuming here that this is the common mechanism through which they exert toxic effects. The suitability of the CA model is also supported by its argued higher conservativeness in environmental assessment compared to the alternative model for mixture toxicity of Independent Action that assumes dissimilar modes of action of the mixture components (Cedergreen et al., 2008). CA is mathematically represented in Eq. (1), where C*i* represents the individual concentrations of each *i* component present in the mixture with a total effect of x % and ECx*i* are those concentrations of the components that would alone cause the same effect x*i* as observed for the mixture.

$$\sum_{i=1}^{n} \frac{Ci}{ECx_i} = 1 \quad (1)$$

As deducible from CA formulation, the toxic strength of a mixture is given by the sum of the quotients Ci/ECx_i (toxic units; TU), which should equal 1 if there are no interactions between the components of the mixture, i.e. if they behave through simple additivity. On this basis, we calculated the TU sum for each DES (TU_{DES}) using the estimated EC_{50} following exposure to each DES and the corresponding starting materials, as detailed in Eq. (2):

$$TU_{DES} = \left(\frac{EC_{50}A\ DES}{EC_{50}A}\right) + \left(\frac{EC_{50}B\ DES}{EC_{50}B}\right) \quad (2)$$

where, A and B represent each component of the mixture, i.e. the starting materials; EC_{50} DES correspond to the EC_{50} values of the starting materials (A or B) dosed as part of the DES (this concentration