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Short Communication

# Are deep eutectic solvents benign or toxic?

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## HIGHLIGHTS

- Many recent studies reported that DESs are non-toxic and benign solvents.
- This is the first time that toxicity and cytotoxicity of DESs were studied.
- We found the cytotoxicity of DESs was much higher than their individual components.
- Toxicity and cytotoxicity of DESs varied depending on the structure of components.
- Careful usage of the terms non-toxicity and biodegradability must be considered.

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## ABSTRACT

In continuation of investigation for environmentally benign protocol for new solvents termed deep eutectic solvents (DESs), it is herein reported results concerning the toxicity and cytotoxicity of choline chloride (ChCl) based DESs with four hydrogen bond donors including glycerine, ethylene glycol, triethylene glycol and urea. The toxicity was investigated using two Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. The cytotoxicity effect was tested using the *Artemia salina* leach. It was found that there was no toxic effect for the tested DESs on all of the studied bacteria confirming their benign effects on these bacteria. Nevertheless, it was found that the cytotoxicity of DESs was much higher than their individual components (e.g. glycerine, ChCl) indicating that their toxicological behavior is different. For our best knowledge this is the first time that toxicity and cytotoxicity of DESs were studied. The toxicity and cytotoxicity of DESs varied depending on the structure of components. Careful usage of the terms non-toxicity and biodegradability must be considered. More investigation on this matter is required.



## 1. Introduction

In this era of chemical synthesis, the improvement of reaction efficiency, avoidance of toxic reagents, reduction of waste, and the responsible utilization of resources have become critical objectives (Disale et al., 2012). Therefore, seeking green solvents, catalysts and media for chemical reactions and processes are of great interest.

DESs are currently attracting widespread scientific and technological interest as low cost alternative to conventional ionic liquids (ILs). In principle, DES is a mixture of two or more compounds which has a melting point lower than that of either of its components (Abbott et al., 2004; Hayyan et al., 2010). DESs possess many advantages compared to ILs such as: (1) they are simple to synthesize since the components salt and hydrogen bond donor (HBD)/complexing agent can be easily mixed and converted to DES without need for further purification; (2) they have low production cost due to the low cost of raw materials; and (3) DES is expected to have good biocompatibility when using quaternary ammonium salts such as choline chloride (ChCl) that is being used as an additive in chicken food (Jhong et al., 2009; Singh et al., 2012).

Many recent publications reported that DESs are non-toxic, eco-friendly, biodegradable and benign solvents (Abbott et al., 2004; Jhong et al., 2009; Hayyan et al., 2012; Singh et al., 2012; Wu et al., 2012). Lately, the unsafe of inappropriate wording describing IL properties has been recently highlighted such as non-flammability even though ILs are known to thermally decompose at varying temperature, which, like in case of polymers, requires other measurements than flash point to assess the flammability (Diallo

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et al., 2012). In the case of DESs, the careful use of terminology should be taken into consideration since the properties of DESs are still being investigated, even less than ILs, and the main characteristics of DES are not yet studied, e.g. toxicity, biodegradability.

While these solvents show excellent applicability in a wide range of reactions and processes their widespread involvement are limited to biological and industrial applications due to the lack of their corrosivity and toxicological data. In most countries, before any chemical product can be legally supplied, sold, or used it must be registered by the appropriate authority. The DES, being mixtures, are not covered by Regulation (EC) No 1907/2006, called 'REACH directive', even though some companies manufacture them and put them on the market. The registry contains components of DES only.

Recently, Morrison et al. (2009) investigated the potential of utilizing DES as drug solubilization vehicles for oral dosing for rats during early development pharmacokinetic investigations. They concluded that DES can be a promising vehicle for increasing exposure of poorly soluble compounds in pre-clinical studies. However, their assumption was based on the components in DES that were pharmaceutically acceptable since the material safety data sheets indicate that the toxicity profile for rats (oral LD 50) for urea, ChCl and malonic acid are 8471 mg $kg^{-1}$, 3400 mg $kg^{-1}$ and 1310 mg $kg^{-1}$, respectively, while the toxicity profile for mice (oral LD 50) for urea, ChCl and malonic acid are 11 000 mg $kg^{-1}$, 3900 mg $kg^{-1}$ and 4000 mg $kg^{-1}$, respectively (Morrison et al., 2009). This hypothesis may be incorrect since it did not take into consideration the possibility of synergetic effect of combining the compounds in the DES. Basically, synergetic effect is the effect arising between two or more substances, entities, agents or factors, which is greater than the sum of their individual effects. Furthermore, the investigation involved only the solubility of such drug compounds without assessing the toxicity profile of the formed DES.

In this work, the toxicity of DES using two Gram positive bacteria, i.e. *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram negative bacteria, i.e. *Escherichia coli* and *Pseudomonas aeruginosa* was investigated. These bacteria are among the most common microorganisms that have been used extensively to investigate the toxicity of various chemicals (Jardim et al., 1990; Chen et al., 2010; Fadli et al., 2011; Spaulding et al., 2012; Young et al., 2012; Fu et al., 2013). In addition, the cytotoxicity of the tested DESs towards the *Artemia salina leach* (brine shrimp larvae or hatches) was investigated. The brine shrimp cytotoxicity assay is considered a convenient probe for preliminary assessment of cytotoxicity, detection of mycotoxins, heavy metals, pesticides and testing of dental materials. It can also be extrapolated for anti-tumor activity and cell-line toxicity (Hartl and Humpf, 2000; Manilal et al., 2009). The experimental methodology is described in the Supplementary Information. The tested DESs were based on ChCl combined with four HBDs which are glycerine (Gl), ethylene glycol (EG), triethylene glycol (TEG) and urea (U). These DESs were selected based on their potential use in several important applications as reported by many recent studies (Pollet et al., 2008; Morrison et al., 2009; Hayyan et al., 2010; Lindberg et al., 2010; Wang et al., 2010; Cojocaru et al., 2011; Domínguez de María and Maugeri, 2011; Lloyd et al., 2011; Shahbaz et al., 2011; Guillamat et al., 2012; Ramesh et al., 2012a,b; Rimsza and Corrales, 2012; Wu et al., 2012).

Table 1 illustrates that all investigated DESs have no inhibition on the selected bacteria in this preliminary study. This is in agreement with expectations reported by different research groups (Abbott et al., 2004; Jhong et al., 2009; Singh et al., 2012; Wu et al., 2012).

On the other hand, it was found that there is cytotoxicity for these DESs on brine shrimp hatches, Table 2. To determine if the cause is due to one component or both components, they were tested using the same concentration of each component separately dissolved in distilled water, Table 2. The results show that all studied DESs have higher potent cytotoxicity than their individual components. To determine if the increase in cytotoxicity is due to the presence of the salt and HBD together, i.e. synergetic effect, or because of formation of the DES, the following experiments were conducted. Pre-determined amounts of the salt and HBD were added to distilled water to form a solution having the same concentration as that of the aqueous solution of the respective DES. The cytotoxicity of the resulting solution was determined under the same conditions. The results are listed in Table 3. It is very clear from Table 3 that there is no significant synergetic effect due to the presence of salt and HBD. That may be due to the way of aqueous

**Table 1**
DES influence on bacteria inhibition.

| Bacteria | ChCl:Gl | Gl | ChCl |
|---|---|---|---|
| *Escherichia coli* | NI[a] | NI | NI |
| *Staphlococcus aureus* | NI | NI | NI |
| *Pseudomonas aeuriginosa* | NI | NI | NI |
| *Bacilus subtilis* | NI | NI | NI |
| Bacteria | ChCl:EG | EG | ChCl |
| *Escherichia coli* | NI | NI | NI |
| *Staphlococcus aureus* | NI | NI | NI |
| *Pseudomonas aeuriginosa* | NI | NI | NI |
| *Bacilus subtilis* | NI | NI | NI |
| Bacteria | ChCl:TEG | TEG | ChCl |
| *Escherichia coli* | NI | NI | NI |
| *Staphlococcus aureus* | NI | NI | NI |
| *Pseudomonas aeuriginosa* | NI | NI | NI |
| *Bacilus subtilis* | NI | NI | NI |
| Bacteria | ChCl:U | U | ChCl |
| *Escherichia coli* | NI | NI | NI |
| *Staphlococcus aureus* | NI | NI | NI |
| *Pseudomonas aeuriginosa* | NI | NI | NI |
| *Bacilus subtilis* | NI | NI | NI |

[a] NI: No inhibition.

**Table 2**
The cytotoxicity of DES towards brine shrimp (min).

| Number of nauplii[a] | DES<br>ChCl:Gl | HBD solution<br>Gl | Salt solution<br>ChCl |
|---|---|---|---|
| 2 | 0.15 ± 0.05 | 5.53 ± 0.49 | 10.30 ± 1.13 |
| 4 | 0.44 ± 0.05 | 6.23 ± 0.26 | 18.56 ± 1.48 |
| 6 | 1.01 ± 0.02 | 7.54 ± 0.54 | 22.11 ± 1.50 |
| 8 | 3.20 ± 0.12 | 10.09 ± 0.99 | 25.01 ± 1.29 |
| 10 | 5.32 ± 0.03 | 13.04 ± 0.72 | 40.37 ± 0.79 |
| | ChCl:EG | EG | ChCl |
| 2 | 1.59 ± 0.41 | 10.18 ± 0.67 | 13.52 ± 0.43 |
| 4 | 3.12 ± 0.62 | 26.92 ± 1.41 | 21.33 ± 0.83 |
| 6 | 5.55 ± 0.42 | 33.54 ± 0.43 | 30.37 ± 0.75 |
| 8 | 8.46 ± 0.49 | 44.33 ± 0.23 | 36.38 ± 1.77 |
| 10 | 10.13 ± 0.88 | 53.24 ± 1.72 | 39.54 ± 1.31 |
| | ChCl:TEG | TEG | ChCl |
| 2 | 0.20 ± 0.04 | 10.22 ± 0.17 | 16.53 ± 0.06 |
| 4 | 0.35 ± 0.06 | 13.33 ± 0.15 | 22.41 ± 0.10 |
| 6 | 0.56 ± 0.03 | 16.17 ± 0.21 | 27.43 ± 0.11 |
| 8 | 1.50 ± 0.11 | 21.23 ± 0.30 | 32.23 ± 0.29 |
| 10 | 2.58 ± 0.26 | 26.12 ± 0.13 | 33.49 ± 0.08 |
| | ChCl:U | U | ChCl |
| 2 | 0.45 ± 0.05 | 22.18 ± 0.03 | 18.20 ± 0.06 |
| 4 | 1.35 ± 0.08 | 26.10 ± 0.64 | 24.23 ± 0.09 |
| 6 | 1.56 ± 0.11 | 34.26 ± 0.43 | 29.31 ± 0.75 |
| 8 | 2.30 ± 0.15 | 48.45 ± 0.17 | 35.26 ± 0.37 |
| 10 | 4.05 ± 0.27 | 52.35 ± 0.56 | 47.28 ± 3.32 |

[a] Nauplii: The free-swimming first stage of the larva of Brine shrimp (*Artemia salina*).

**Table 3**
The cytotoxicity of components forming DESs dissolved simultaneously in water.

| Number of nauplii | Time (min) | | | |
|---|---|---|---|---|
| | ChCl:Glaq[a] | ChCl:EGaq | ChCl:TEGaq | ChCl:Uaq |
| 2 | 20.27 ± 0.23 | 15.49 ± 0.49 | 18.40 ± 0.12 | 25.37 ± 0.17 |
| 4 | 30.26 ± 0.16 | 25.34 ± 0.22 | 25.42 ± 0.10 | 40.24 ± 0.26 |
| 6 | 35.56 ± 0.41 | 37.59 ± 0.40 | 29.41 ± 0.16 | 53.59 ± 0.73 |
| 8 | 48.03 ± 0.43 | 43.33 ± 0.21 | 35.56 ± 0.48 | 64.37 ± 0.16 |
| 10 | 53.03 ± 0.37 | 49.10 ± 0.37 | 42.26 ± 0.72 | 74.54 ± 0.51 |

[a] ChCl:HBDaq: Components of DES dissolved in distilled water to form a solution having the same concentration of DES shown in Table 2.

solution preparation by adding individual components simultaneously to the distilled water in the same ratio of DES, but no DES was prepared prior to this dilution.

This shows that the hydrogen bonding between the HBD and the anion of the salt that forms the DES does not only affect the physical properties of the pure components but it also affects the chemical structure of the mixture. Specifically, *A. salina* leaches are susceptible to DESs toxicity while they are resistant to the toxicity of aqueous solutions for DES components. The viability of *A. salina* leaches is very limited, as stated in Table 2, where 10 cells do not survive for longer than 5.32 min in ChCl:Gl, 10.13 min in ChCl:EG, 2.58 min in ChCl:TEG and 4.05 min in ChCl:U DESs.

There are other possible reasons that can also cause the cytotoxicity for brine shrimps such as the lack of oxygen or the difficulty in movement due to the high viscosity of DES. The chemical nature of the DES components affects the viscosity of DES (e.g. the type of salt and HBD, molar ratio), water content and temperature (Abbott et al., 2006; Zhang et al., 2012).

However, further studies are required and recommended for better understanding of the interaction and complexing nature of DES compositions. Based on these preliminary results, DESs are expected to serve as selective chemicals which may have the potential to be destructive for certain types of cells, e.g. any abnormal cells (tumor cells) and non-destructive for other cells. Therefore, the investigation of toxicity for human and animal cells is essential for future studies. The question is now raised: since chemotherapy and radiotherapy have serious side effects, would DESs be potential alternative drugs or at least drugs vehicles or even both? If yes, this will be a real breakthrough.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.11.004.

## References

Abbott, A.P., Boothby, D., Capper, G., Davies, D.L., Rasheed, R.K., 2004. Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids. J. Am. Chem. Soc. 126, 9142–9147.

Abbott, A.P., Capper, G., Gray, S., 2006. Design of improved deep eutectic solvents using hole theory. Chem. Phys. Chem. 7, 803–806.

Chen, H., Yao, J., Wang, F., Zhou, Y., Chen, K., Zhuang, R., Choi, M.M.F., Zaray, G., 2010. Toxicity of three phenolic compounds and their mixtures on the Gram-positive bacteria *Bacillus subtilis* in the aquatic environment. Sci. Total Environ. 408, 1043–1049.

Cojocaru, P., Magagnin, L., Gomez, E., Vallés, E., 2011. Using deep eutectic solvents to electrodeposit CoSm films and nanowires. Mater. Lett. 65, 3597–3600.

Diallo, A.O., Len, C., Morgan, A.B., Marlair, G., 2012. Revisiting physico-chemical hazards of ionic liquids. Sep. Purif. Technol. 97, 228–234.

Disale, S.T., Kale, S.R., Kahandal, S.S., Srinivasan, T.G., Jayaram, R.V., 2012. Choline chloride·2ZnCl2 ionic liquid: an efficient and reusable catalyst for the solvent free Kabachnik–Fields reaction. Tetrahedron Lett. 53, 2277–2279.

Domínguez de María, P., Maugeri, Z., 2011. Ionic liquids in biotransformations: from proof-of-concept to emerging deep-eutectic-solvents. Curr. Opin. Chem. Biol. 15, 220–225.

Fadli, M., Chevalier, J., Saad, A., Mezrioui, N.-E., Hassani, L., Pages, J.-M., 2011. Essential oils from moroccan plants as potential chemosensitisers restoring antibiotic activity in resistant gram-negative bacteria. Int. J. Antimicrob. Agents 38, 325–330.

Fu, M., Qin, C., Li, W., Yan, Y., Zeng, L., Yang, X., 2013. Effect of glucosamine and chitooligomer on the toxicity of arsenite against *Escherichia coli*. Carbohydr. Polym. 91, 390–393.

Guillamat, P., Cortés, M., Vallés, E., Gómez, E., 2012. Electrodeposited CoPt films from a deep eutectic solvent. Surf. Coat. Technol. 206, 4439–4448.

Hartl, M., Humpf, H.U., 2000. Toxicity assessment of fumonisins using the brine shrimp (Artemia salina) bioassay. Food Chem. Toxicol. 38, 1097–1102.

Hayyan, M., Mjalli, F.S., Hashim, M.A., AlNashef, I.M., 2010. A novel technique for separating glycerine from palm oil-based biodiesel using ionic liquids. Fuel Process. Technol. 91, 116–120.

Hayyan, A., Mjalli, F.S., AlNashef, I.M., Al-Wahaibi, T., Al-Wahaibi, Y.M., Hashim, M.A., 2012. Fruit sugar-based deep eutectic solvents and their physical properties. Thermochim. Acta 541, 70–75.

Jardim, W.F., Pasquini, C., Guimarães, J.R., de Faria, L.C., 1990. Short-term toxicity test using *Escherichia coli*: monitoring $CO_2$ production by flow injection analysis. Water Res. 24, 351–354.

Jhong, H.-R., Wong, D.S.-H., Wan, C.-C., Wang, Y.-Y., Wei, T.-C., 2009. A novel deep eutectic solvent-based ionic liquid used as electrolyte for dye-sensitized solar cells. Electrochem. Commun. 11, 209–211.

Lindberg, D., de la Fuente Revenga, M., Widersten, M., 2010. Deep eutectic solvents (DESs) are viable cosolvents for enzyme-catalyzed epoxide hydrolysis. J. Biotechnol. 147, 169–171.

Lloyd, D., Vainikka, T., Murtomäki, L., Kontturi, K., Ahlberg, E., 2011. The kinetics of the $Cu^{2+}/Cu^{+}$ redox couple in deep eutectic solvents. Electrochim. Acta 56, 4942–4948.

Manilal, A., Sujith, S., Seghal Kiran, G., Selvin, J., Shakir, C., 2009. Cytotoxic potentials of red alga, laurencia brandenii vollected from the Indian Coast. Global J. Pharmacol. 3, 90–94.

Morrison, H.G., Sun, C.C., Neervannan, S., 2009. Characterization of thermal behavior of deep eutectic solvents and their potential as drug solubilization vehicles. Int. J. Pharm. 378, 136–139.

Pollet, B.G., Hihn, J.-Y., Mason, T.J., 2008. Sono-electrodeposition (20 and 850 kHz) of copper in aqueous and deep eutectic solvents. Electrochim. Acta 53, 4248–4256.

Ramesh, S., Shanti, R., Morris, E., 2012a. Exerted influence of deep eutectic solvent concentration in the room temperature ionic conductivity and thermal behavior of corn starch based polymer electrolytes. J. Mol. Liq. 166, 40–43.

Ramesh, S., Shanti, R., Morris, E., 2012b. Studies on the plasticization efficiency of deep eutectic solvent in suppressing the crystallinity of corn starch based polymer electrolytes. Carbohydr. Polym. 87, 701–706.

Rimsza, J.M., Corrales, L.R., 2012. Adsorption complexes of copper and copper oxide in the deep eutectic solvent 2:1 urea–choline chloride. Comput. Theor. Chem. 987, 57–61.

Shahbaz, K., Mjalli, F.S., Hashim, M.A., AlNashef, I.M., 2011. Eutectic solvents for the removal of residual palm oil-based biodiesel catalyst. Sep. Purif. Technol. 81, 216–222.

Singh, B.S., Lobo, H.R., Shankarling, G.S., 2012. Choline chloride based eutectic solvents: magical catalytic system for carbon–carbon bond formation in the rapid synthesis of β-hydroxy functionalized derivatives. Catal. Commun. 24, 70–74.

Spaulding, A.R., Lin, Y.-C., Merriman, J.A., Brosnahan, A.J., Peterson, M.L., Schlievert, P.M., 2012. Immunity to Staphylococcus aureus secreted proteins protects rabbits from serious illnesses. Vaccine 30, 5099–5109.

Wang, S.-M., Chen, W.-L., Wang, E.-B., Li, Y.-G., Feng, X.-J., Liu, L., 2010. Three new polyoxometalate-based hybrids prepared from choline chloride/urea deep eutectic mixture at room temperature. Inorg. Chem. Commun. 13, 972–975.

Wu, S.-H., Caparanga, A.R., Leron, R.B., Li, M.-H., 2012. Vapor pressure of aqueous choline chloride-based deep eutectic solvents (ethaline, glyceline, maline and reline) at 30–70 °C. Thermochim. Acta 544, 1–5.

Young, Y.-F., Lee, H.-J., Shen, Y.-S., Tseng, S.-H., Lee, C.-Y., Tai, N.-H., Chang, H.-Y., 2012. Toxicity mechanism of carbon nanotubes on *Escherichia coli*. Mater. Chem. Phys. 134, 279–286.

Zhang, Q., De Oliveira Vigier, K., Royer, S., Jerome, F., 2012. Deep eutectic solvents: syntheses, properties and applications. Chem. Soc. Rev. 41, 7108–7146.