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Toxicity evaluation of selected ionic liquid compounds on embryonic development of Zebrafish

Nadin Younes[a,1], Rola Salem[a,b,1], Maha Al-Asmakh[a,b], Tausif Altamash[c], Gianfranco Pintus[a,b], Majeda Khraisheh[c], Gheyath K. Nasrallah[a,b,*]

[a] Department of Biomedical Science, College of Health Sciences, Qatar University, Women's Science building, C01, P.O Box: 2713, Doha, Qatar
[b] Biomedical Research Center, Qatar University, P.O Box: 2713, Doha, Qatar
[c] Department of Chemical Engineering, College of Engineering, Qatar University, P.O Box: 2713, Doha, Qatar

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ABSTRACT

Hydrate formation in seafloor pipelines is considered an economic and flow assurance issue for the oil and gas industries. Ionic liquids (ILs) have been recently used as potential hydrate inhibitors. Although branded as green compounds, their ecotoxicity in case of leakage from pipelines onto the aquatic environment needs more deep evaluations. Here, we investigate the impacts of three ILs previously used as successful thermodynamic hydrate inhibitors namely choline chloride (ChC1), 1-methyl-1-propyl pyrrolidinium triflate (PMPy [triflate]) and tetramethyl ammonium acetate (TMAA). Mortality (including LC50), teratogenicity, locomotion and neurotoxicity, and hatching rate were utilized to investigate any potential acute toxicity of these ILs on embryonic development of zebrafish. No significant mortality or teratogenic effects were found for all tested compounds in a concentration range between 50 and 200 mg/L. The LC50 was significantly higher than the tested dose > 200 mg/L. While, up to 200 mg/L all compound had no impact on the survival rate, ChCl showed a significant effect on neuromuscular development as judged by the increase of spontaneous tail coiling activity (25 VS 4 burst/minutes of the negative control-treated embryos). Further, apart from PMPy [triflate], ChC1 and TMAA had a significant adverse effect on the hatching rate of the treated embryos at concentrations of 200 mg/L. However, this effect was very mild at lower concentrations (≤ 100 mg/L). Our data indicate that within the tested concentrations both TMAA and PMPy [triflate] had no or little potential harmful effect on embryonic development of aquatic fauna “green”, while ChC1 should be used with caution.

1. Introduction

Ionic liquids, salts in the liquid state, have attracted considerable attention in various application due to their unique properties and characteristics as well as for their abilities to be tailor-made to suit various applications in comparison with conventional solvents (Greaves, 2015; Egorova et al., 2017; Vekariya, 2017). Amongst the most useful properties are their negligible volatility, broad liquid range vast electrochemical windows, high thermal stability, recyclability, and their physical or transport properties that can be tuned by appropriately chosen cation–anion pair for a specific function (Welton, 1999; Rogers and Seddon, 2003; MacFarlane et al., 2016). Because of their favorable properties, ILs have been the focus of many published research regarding a wide variety of applications such as gas separation technology, fuels cell, batteries and gas hydrate inhibition (Greaves, 2015).

Gas hydrates are crystalline solid formations that have major economic (10–15% of production costs) (Emmanuel, 2012) and operating implications for flow assurance operators in the oil and gas industries. The combination of high pressure, low temperature and the presence of appropriately sized “guest” molecule such as methane, ethane, and propane, is at the basis of gas hydrates formation in seabed gas pipelines. In this context, altering any of the conditions (thermodynamic or kinetic) that is required for the formation of these gas hydrates can cause a delay or inhibition in its formation. For this reason, finding environmental friendly “green”, cost effective gas hydrates inhibitors is of great interest to oil and gas operating companies associated with deep-sea excavation. A number of chemicals have been used to inhibit hydrates formation with two main drawbacks related to (i) cost associated with the use of large quantities and (ii) the chemical solvents and its ecotoxicity [reviewed in (Finšgar and Jackson, 2014)]. Accordingly,

* Corresponding author at: Department of Biomedical Science, College of Health Sciences, Qatar University, P.O Box: 2713, Doha, Qatar.
E-mail addresses: ny1204022@qu.edu.qa (N. Younes), rs1202653@qu.edu.qa (R. Salem), maha.alasmakh@qu.edu.qa (M. Al-Asmakh), Tausif.AlTamash@qu.edu.qa (T. Altamash), gpintus@qu.edu.qa (G. Pintus), m.khraisheh@qu.edu.qa (M. Khraisheh), gheyath.nasrallah@qu.edu.qa (G.K. Nasrallah).
[1] **Equal Contributions**.

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ILs gained momentum as potential hydrate inhibitors due to their “designer” nature and green label.

Nevertheless, studies concerning the direct impact of ILs on living organism are scarce and only incomprehensive works reported the toxicity of ILs on aquatic ecosystem and living aquatic organisms such as *Daphnia magna*, *Danio rerio*, *Poecilia reticulate*, *Cephalopholis cruentata, Latescalcarifer, Carassius auratus* etc (Başer et al., 2003; Bernot et al., 2005; Pretti et al., 2006; Samorì et al., 2007; Pretti et al., 2009; Wang et al., 2010a; El-Harbawi, 2014; El-Harbawi, 2014; El-Harbawi, 2014; Ruokonen et al., 2016; Thamke and Kodam, 2016).

Recently, the ability of some ILs to act as gas hydrate inhibitors have been investigated by our collaborators (Qureshi et al., 2016; Tariq et al., 2016a, 2016b; Altamash et al., 2017; Mohamed, 2017). In this context, we realized that studies concerning the ecotoxicity of some of the ILs reported in the above-mentioned study are lacking. Therefore, the present study has been undertaken to investigated potential toxic effects of three different type of ILs, such as ChCl, PMPy [triflate] and TMAA, using zebrafish (*Danio rerio*) embryo as a model for marine fauna toxicity. We decided to specifically investigate the toxicity of the three selected compounds since they showed the most promising results as gas hydrate inhibitors (Qureshi et al., 2016; Tariq et al., 2016a; Tariq et al., 2016b; Altamash et al., 2017; Mohamed, 2017). Since no toxicity studies have been performed on these selected compounds, we investigated a wider range of concentrations (50, 100, 200 mg/L) in order to find both the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). In this regard, the select concentrations are consistent with previously published work using other ILs (Perez et al., 2017; Ruokonen et al., 2016) and within the toxicity rating scale provided by the U.S. Fish and Wildlife Service (USFWS) as previously presented (El-Harbawi, 2014).

2. Materials and methods

2.1. Chemicals

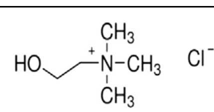
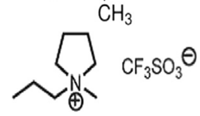
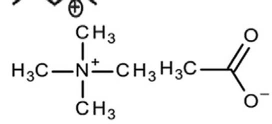
ILs have been purchased from IoLiTec GmBH, Germany. The structure and other details of ILs are depicted in Table 1. Diethylaminobenzaldehyde (DEAB) (Sigma, Germany) is a competitive inhibitor of aldehyde dehydrogenases known to generate toxic and teratogenic effects in zebrafish, and was therefore used as positive toxicity control in our experimentation. N-Phenylthiourea (PTU) (Sigma, Germany) was used to inhibit pigment formation in the developing zebrafish embryos to facilitate their visualization under the microscope. Stock solutions for zebrafish embryos experiments such as DEAB, PTU, Egg water, PBS, and methylene blue solution was prepared as described in (Nasrallah et al., 2018; Rasool et al., 2018). A stock solution 1.0 mg/mL for the three ILs ChCl, PMPy [triflate] and TMAA was prepared by adding 10 mg of each IL to 10 mL 1XPBS and vortexed several times to make sure that the ILs were dissolved completely. For toxicity experiment, ILs were further diluted in zebrafish Egg medium to the desired tested concentration (50, 100, 200 mg/L).

2.2. Zebrafish culture

Wild-type zebrafish embryos (AB strain) were used for this experimentation. The AB zebrafish were originally purchased in 2014 from Model Fish Facility (MFU), Norwegian University of Life Sciences, Department of Production Animal clinical Sciences, Oslo, Norway. The fish colonies were then maintained and expanded in our Pentair, Z-Hab Duo Systems, Inc, USA (located at the Biomedical Research Center, Qatar University). The fish were left in a 14 h light/10 h dark cycle with a water temperature of [28]°C (Korenbrot et al., 2013). Adult zebrafish were fed with commercially available brine shrimps and/ or dry food twice a day (morning and late afternoon meals). Our zebrafish aquarium system (Pentair, Z-Hab Duo Systems, Inc, USA) is a semiautomated system that is provided by different probes (sensors) and continuous monitoring system and a dashboard showing the level conductivity, temperature, pH. Our aquatic system is also provided with automatic dosing system for sodium bicarbonate. The system is continuously fed by a reverse osmosis water (RO) (a RiOs™ MERCK, Germany). In addition, regular water exchanges are also made automatically by the Z-Hab Duo Systems to keep these ions within the acceptable range. Further the system is provided by different type of mechanical recirculating filtration and activated carbon filters to adsorb volatile organics and other contaminants and optimize water clarity for effective UV disinfection. Further, the level of ammonia is also maintained by growing nitrate reducing bacteria in the sump bacteria (added to the system once at the time of insulation) that convert ammonia into nitrite. This bacterium is not toxic to the fish and is commonly used by plants or/or algae in the aquarium. Further, to ensure that the biological filter is working efficiently, weekly checking of the ammonia and nitrite levels in the aquarium are made using a commercial kit (JBL, Germany). This biological filtration system guarantee that ions such as ammonia, nitrate, and nitrite are maintained below the detectable limits (detectable limits expressed in mg/mL are 0.05–0.1 for ammonia, 0.5–10 for nitrate and 0.01–0.025 nitrite).

Fish were prepared for mating by placing male and female adult fish overnight in the mating tank separated by the tank divider and left in the dark. The dividers were removed in the morning and the fish were left to mate for 5 h. Fertilized eggs were collected every 1 or 2 h, then viable eggs were selected and washed with PTU solution before use for the experiments. All animal experiments were carried out according to national and international guidelines for the use of zebrafish in experimental settings (Reed and Jennings, 2011) and in accordance with the animal protocol guidelines required by the Qatar University in laboratory animal and Policy on Zebrafish Research established by Department of Research in the Ministry of Public Health, Qatar (Ministry of Public Health, 2017).

Table 1
Common name, abbreviation, molecular weight, structure, referred purity and concentrations of the ILs studied in this work.

Ionic liquids (ILs)	Abbreviation	Molecular weight (g/mol)	Chemical structure	Purity, [Ref.]	Tested concentration (mg/L)
Choline Chloride	**ChCl**	**140**		97% (Mohamed et al., 2017)	**50, 100, 200**
1-Methyl-1-Propylpyrrolidinium Triflate	**PMPy [Triflate]**	**277**		**98%** (Qureshi et al., 2016)	**50, 100, 200**
Tetramethylammonium acetate	**TMAA**	**133**		~97% (Tariq et al., 2016b)	**50, 100, 200**