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Cytotoxicity of protic ionic liquids towards the HaCat cell line derived from human skin

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ABSTRACT

In this work we have investigated the toxicity of 10 PILs, consisting of ethyl-, ethanol-, diethanol- and triethanolammonium cations paired with nitrate, formate, acetate and glycolate anions. Their toxicity was quantified by the EC_{50} values of each of these PILs towards HaCat cells, which are derived from human skin cells. Additional salts and solvents were used for comparison including DMSO, choline chloride, potassium nitrate, sodium acetate and ethanol to distinguish if the toxicity changes were due to ionicity, short chain amphiphilic behaviour, or specific ion effects. The toxicity followed the general trend of choline chloride < acetate containing PILs < DMSO < sodium acetate < ethanol < nitrate containing PILs or salt. Ethanolammonium acetate and ethylammonium acetate were identified as having the lowest toxicities of the PILs, being slightly more toxic than choline chloride or DMSO. Overall the toxicity was found to be highly dependent on the cation and anion combination, with the anion having a stronger affect. It was evident that the PILs can be tailored to vary their toxicities, and this is expected to be dependent on which cell lines are used.

1. Introduction

Ionic liquids (ILs) are salts with low melting points, typically <100 °C. They are highly tailorable solvents whose physicochemical, thermal and solvent properties can be modified through varying the structure of the cation and/or anion. Similarly, their toxicity and environment impact are also dependent on which ions are present [1–4]. While they are often referred to as 'green' solvents due to their negligible vapour pressure, stability and generally low flammability, it may be better to describe them as greener when compared to organic molecular solvents [1,2,5]. Protic ionic liquids (PILs) are a subset of ILs formed through the neutralisation reaction of a Brønsted acid and a Brønsted base, and hence are simple to synthesis, and generally cheaper than aprotic ILs [6,7]. A few PILs have been reported to have potentially good biocompatibility [8,9], and some studies have shown lower toxicities with PILs compared to their aprotic counterparts [10–12]. The toxicity and potential environmental impact of PILs is expected to be lower than those of AILs due to their simpler structure. However, the industrial use of ILs requires a good understanding of their toxicity [10,13], and with very little reported on the cytotoxicity of PILs, this limits their use in potential biomedical applications. For example, in many applications low toxicity is desirable such as for stabilising proteins and cell cryopreservation, however some applications require selective toxicity, such as in cancer treatment, and in the extraction of bioactive molecules from cells [13,14]. A few studies have shown that the toxicity and environmental impact observed that PILs consisting of aliphatic ammonium cations paired with organic anions were considered non-toxic for aquatic ecotoxicity tests, and terrestrial ecotoxicity [11,12,15]. However, other studies also showed that pyridinium based PILs were toxic in aquatic ecotoxicity tests [16,17].

By contrast, there has been considerable toxicity data reported for aprotic ILs, though direct comparison between studies is difficult due to the usage of different cell lines and different ILs. Their toxicity is highly dependent on which cation and anion is present, and in general the lowest toxicity is observed for choline chloride. Some important findings are that the toxicity of 1-n-butyl-3-methylimidazolium containing ILs towards the HeLa cell line, which is the most commonly used human cell derived from cervical cancer, showed a strong anion dependence [18]. Previous studies have explored the cellular toxicity of ILs, on human keratinocyte and fibroblast cells [19], human corneal epithelial cells [20], and breast cancer cells [21], however this has only been reported for aprotic ILs. In a study exploring different cations, the imidazolium-based ILs were found to be the most toxic towards fungi, followed by pyridinium-, pyrrolidinium-, and piperidinium-based ILs, whereas cholinium-based ILs were the least toxic [22]. Similarly, it has been reported that the toxicity of alkyltributylphosphonium chloride ILs towards fungus *Aspergillus nidulans* led to membrane damage of the majority of the cells where the alkyl group had more than four carbons. While we note that there is a significant difference between human and fungal cells, with differences in membrane thickness and permeability rates, there are similar trends in the effect of IL structure

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on the extent of cell damage [23]. From these studies it was determined that increasing the alkyl group chain length and/or increasing the number of alkyl groups substituted on the cation ring led to an increase in toxicity, and that varying the pyridium anion significantly altered the toxicity [24]. Microbial based studies on the biological effects of alkylimidazolium ILs to *Vibrio fischeri*, which are rod shaped bacterium, showed high toxicity values ($\log EC_{50}$/μM, 0.182 to 3.94), which places them as more toxic than the molecular solvents of acetone, acetonitrile, methanol, or methyltetrabutylether ($\log EC_{50}$/μM, 3.89 to 7) [25]. A study investigating the toxicity of herbicidal ionic liquids concluded that the hydrophobicity and toxic effects of ILs on living organisms could be decreased through modification of the cation and anions, enabling the design of effective herbicides with reduced ecotoxicity [26]. For the related deep eutectic solvents (DES) consisting of ammonium salts and carboxylic acids, the interactions between the cell membrane and the DES formed led to the disruption of membrane physiological function, causing leakage of cellular content, and consequently cell death [27]. Overall these studies show that the toxicity is highly dependent on the specific IL and the type of cells and organisms it is tested on. Consequently, there is a need for systematic studies to gain greater understanding of the toxicity, particularly on human cells. It is also critical to understand the toxicity of the ILs across a broader range of cells to have insight into their effect on the environment, and their toxicity to living organisms. While there is significant understanding about the toxicity of some aprotic ILs, there has been very little reported for PILs, despite the increasing interest in them as solvents for biological media, and many other applications [6,7].

In aqueous solutions ILs are effectively salts but are often used at significantly higher concentrations than conventional salts. It is well known that salts effect toxicity, even though they are typically present in low concentrations as buffers in the media. The cytotoxic effects of sodium acetate on human skin cells has been extensively studied, and previously it was reported that incubation with 50 mM sodium acetate for 24 h led to a decrease of up to 70% cell viability compared to that seen in cells treated with 1 wt% Triton X-100 as control [28]. Similarly, sodium acetate induced cell differentiation and apoptosis in a colorectal cancer cell line [29]. A study performed on stomach adenocarcinoma cell lines using sodium acetate showed dose-dependency with a decrease in cell growth above 12.5 mM [30]. The *in vitro* cytotoxic effects of saccharin on the transformed rat-bladder epithelial cell line using AY-27 cells was seen to be dependent on the salt, of sodium, potassium and calcium at concentrations of ≥50 mM, where the salt decreased viability [31].

The effect of short chained amphiphilic molecular solvents on cell toxicity has also been considered, since ILs are often amphiphilic. Ethanol is of a similar chain length to many of the PILs used in this current study, and generally has a high toxicity. The metabolism of alcohol and ethanol induced toxicity on human stomach cancer cell line has been studied showing that even 0.25 wt% of ethanol was related to cell death [32]. A study on a gastric cell line showed that the metabolism of ethanol was directly related to first stage of gastric cancer [33]. It was shown by analysing the influence of low concentration of ethanol from 0.5 wt% to understand the cell viability, apoptosis on gastric carcinoma cell line showing that even at a low concentration of 0.5 wt% ethanol has high toxicity on the cell line [34]. Ethanol disrupts the physical structure of cell membranes. In particular, the bilayers become more fluid and permeable: ethanol molecules are able to penetrate through the membrane leading to an increased toxic effect [35].

In this investigation we have determined the cytotoxicity of ten protic ionic liquids towards HaCat cell line derived from human skin. The PILs contained ethylammonium, ethanolammonium, diethanolammonium or triethanolammonium cations paired with nitrate, formate, glycolate, or acetate anions, and their structures are shown in Fig. 1. For comparison, choline chloride was included as a representative biocompatible, low toxicity aprotic salt, DMSO as a commonly used solvent additive for cryopreservation, and ethanol as an amphiphilic molecular solvent of similar size to the cations. The salts

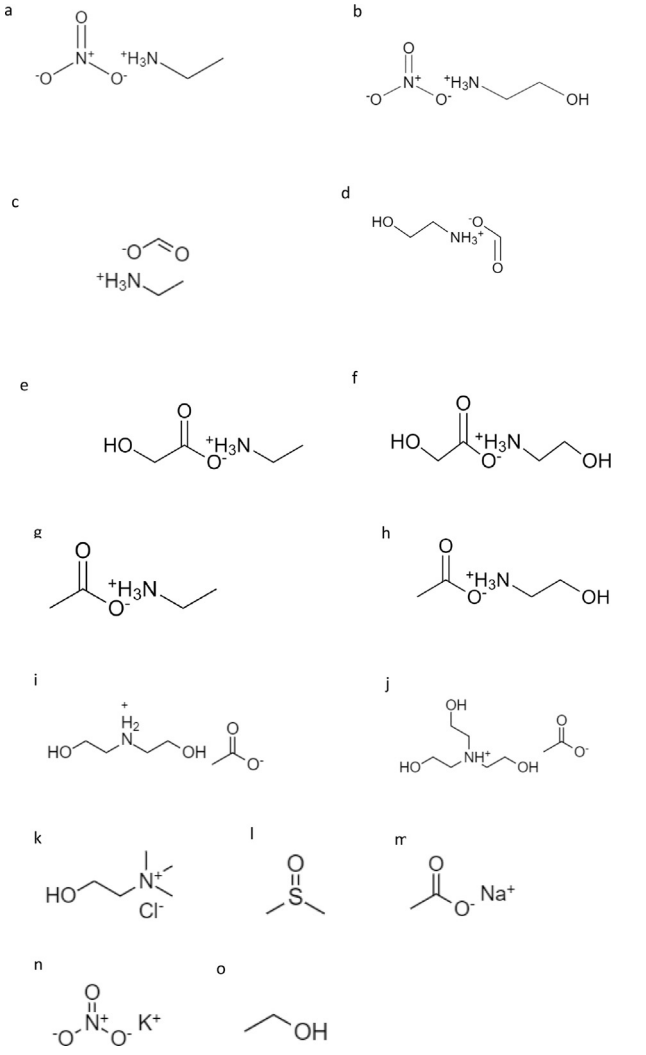

Fig. 1. Chemical structures and abbreviations of the 10 PILs and salts used in this investigation of a) ethylammonium nitrate (EAN), b) ethanolammonium nitrate (EtAN), c) ethylammonium formate (EAF), d) ethanolammonium formate (EtAF), e) ethylammonium glycolate (EAG), f) ethanolammonium glycolate (EtAG), g) ethylammonium acetate (EAA) h) ethanolammonium acetate (EtAA), i) diethanolammonium acetate (DetAA), j) triethanolammonium acetate (TetAA), k) chloline chloride, l) dimethyl sulfoxide, m) Sodium acetate, n) Potassium nitrate, and o) Ethanol.

of potassium nitrate and sodium acetate were included as common salts with the same anions as used in this study. The structures of all the comparison additives are also provided in Fig. 1. The HaCat cell lines derived from human skin was selected as model cells to enable comparison with previous studies, and to understand the toxicity effect towards skin, which is one of the main tissues directly exposed to toxic substances [36]. This is a systematic study designed to address the lack of knowledge about the toxicity of alkylammonium PILs. This study will enable us to compare the toxicity effects of ILs, relative to salts with the same anion, and to molecular solvents with similar chemical structures.

2. Experimental methods

Ethylamine (Sigma-Aldrich, 70 wt%), ethanolamine (Chem Supply, 99%), diethanolamine (Chem Supply, 98%), triethanolamine (Chem Supply, 99%), nitric acid (Chemsupply, 70% *w*/w), acetic acid (Chem Supply, 99%), glycolic acid (Chem Supply, 99%) and formic acid (Merck, 98–100%) were used as received for the synthesis of the PILs. Choline chloride (Sigma-Aldrich, 99 wt%) and dimethyl sulfoxide

(Sigma-Aldrich, 99.7 wt%), sodium acetate (Sigma-Aldrich), potassium nitrate (Sigma-Aldrich) and ethanol (Sigma-Aldrich) were also obtained. The following chemicals were supplied from Life technologies, Dulbecco's Modified Eagle Medium (DMEM), foetal bovine serum (FBS), penicillin-streptomycin (10,000 U/mL), and MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay reagent.

2.1. PIL synthesis and characterisation

The PILs were synthesized by slowly adding equimolar amounts of the acid to the base. The solution was continuously stirred, and the temperature maintained below 10 °C using an ice bath. The water content of the PILs was determined by Karl Fischer Titration, using a Mettler Toledo DL39 Karl Fischer coulometer, and the PILs all had <2 wt% water after synthesis. Proton nuclear magnetic resonance (^{1}H NMR) was taken of each PIL on a Bruker 300 MHz instrument, and these are provided in Fig. S1 of the ESI. The nitrate containing PILs of EAN and EtAN were not included since the absence of protons on the nitrate anion prevented confirmation of the stoichiometry. A Kibron EZPi Du Nuoy surface tensiometer was used to determine the surface tension of the PILs at room temperature (between 21 and 25 °C). The densities of the PILs were measured at 20 °C using a vibrating tube Anton Paar density meter (DMA 4500 M). A SV-A sine wave vibro viscometer was used to determine the dynamic viscosity of the PILs. The kinematic viscosity was obtained by dividing the dynamic viscosity by the density of the respective PILs.

2.2. Cell line

Human skin keratinocyte (HaCaT) cells were used. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), containing 10% foetal bovine serum (FBS) and 1 vol% penicillin-streptomycin (10,000 U/mL). Cells were maintained at 37 °C with 5 vol% CO_2 and 95 vol% relative humidity.

2.3. MTT assay

In vitro cytotoxicity of the ILs was assessed *via* a MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells were seeded at a density of 1×10^5 cells/mL by adding 100 μL per well into 96-well microtiter plates and allowed to incubate for 24 h. The medium was then removed and replaced with 100 μL of IL, salt or molecular solvent solutions at different concentrations up to 7 mM. After 24 h, 10 μL MTT reagent (stock solution of 5.0 mg/mL was prepared in phosphate buffered saline) was added to each well to give a final concentration of 0.5 mg/mL, and the plates incubated at 37 °C for 4 h. The culture medium was then removed by using a pipette and the resulting formazan crystals dissolved in 100 μL of DMSO. The plates were shaken for 30 s, and the optical density measured at 570 nm on a Spectramax Paradigm Molecular devices microplate reader. DMSO and choline chloride was used as positive controls, the untreated cells were used as a negative control, and wells containing complete media with ILs, in the absence of cells, were used as cell-free controls to ensure no interaction of the ILs with the MTT reagent. The EC_{50} values were obtained through linear regression to find the IL, solvent or salt concentrations where there were 50% viable cells.

3. Results

Cytotoxicity measurements for the viability of HaCat cells towards the ten protic ionic liquids (PILs), choline chloride, DMSO, potassium nitrate, sodium acetate and ethanol were determined. HaCat cells are a robust commonly used reproducible cell line, and hence they were chosen to enable comparison to other studies. The chemical structures of the ILs and other salt and solvents added used in this investigation are shown in Fig. 1. Cytotoxicity measurements were made at multiple PIL, solvent and salt concentrations, and are shown in Figs. 2 to 6. The cell viability towards the controls of choline chloride, DMSO and ethanol are shown in Fig. 2. The cell viability for the PILs and salts are grouped relative to the anion, and are shown in Figs. 3–6 for the acetates, formates, glycolates and nitrates, respectively. The EC_{50} values were calculated from these, as the solvent or salt concentration where there were 50% viable cells, and these values are provided in Table 1. These toxicity results are then correlated to the aqueous PIL solution physicochemical properties of density, surface tension and viscosity.

Choline chloride is considered as a highly biocompatible salt [27], and DMSO is used routinely in biological applications such as cryopreservation, despite being relatively toxic [37]. Due to their frequent use with biological molecules, choline chloride and DMSO were used as controls in this study. Similarly, ethanol was included as an amphiphilic molecular solvent with a similar alkyl chain length to the PIL cations. The cell viability in these solvents at different solvent concentrations is provided in Fig. 2, and their EC_{50} values in Table 1. We observed that choline chloride had a higher EC_{50} value of 3.2 mM compared to the EC_{50} of 2.8 mM of DMSO, indicating the choline chloride is less toxic.

The cell viability relative to the PIL concentration is provided in Figs. 3 to 6, for the acetates, formates, glycolates and nitrates, respectively. It is evident from these Figures and Table 1 that the anion had a significant effect on the toxicity. Of the 10 PILs studied, those with acetate anions in general had the lowest toxicities, followed by the glycolates. The cell viability *versus* PIL concentration for the acetate and glycolate containing PILs are relatively linear, as shown in Figs. 3 and 4, and are similar to the plots observed for choline chloride and DMSO. EtAA and EAA were identified as the least toxic PILs towards these cells, with EC_{50} values of 2.5 mM and 2.4 mM respectively, placing them as slightly more toxic than choline chloride and DMSO. In contrast, the formates had an EC_{50} ~0.4 mM and nitrate containing PILs both had EC_{50} well below 0.4 mM, with the nitrates significantly more toxic than the formates, which can be seen from comparing Figs. 5 and 6. Interestingly, the comparison salt of sodium acetate was more toxic than any of the acetate containing PILs, whereas the potassium nitrate was less toxic than either EAN or EtAN. The toxicity of the ethanol was greater than most of the PILs towards the HaCat cells, with the exception of the nitrate PILs.

The cations had less of an effect on the toxicity, with changes in the cations structure not having as dramatic effect on the toxicity. In general, the ethanolammonium cation was slightly less toxic than the ethylammonium cation, with the exception of the acetates where they were similar across the concentration range used. This is evident from Table 1, and also from the cell viability plots. The effect of multiple substitution on the cation was explored, taking the least toxic EtAA and comparing to DEtAA and TEtAA. It can be seen from the EC_{50} values in Table 1 and in Fig. 3, that increasing the number of ethylhydroxyl groups

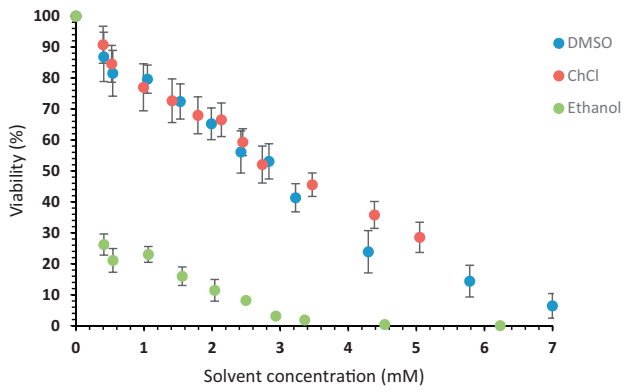

Fig. 2. Viability of HaCat cells towards choline chloride, DMSO and ethanol.

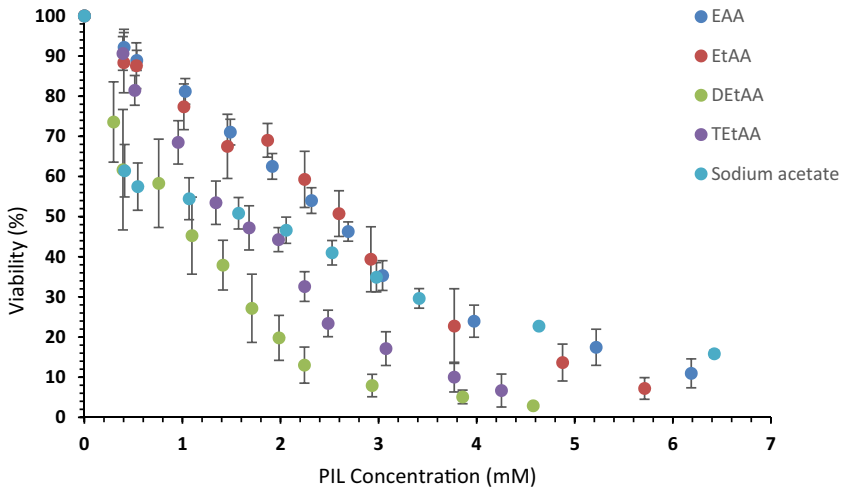

Fig. 3. Viability of HaCat cells towards EAA, EtAA, DtEAA, TEtAA and sodium acetate.

on the ammonium cation affects the toxicity, with EtAA and EAA the least toxic, TEtAA then DEtAA for these HaCat cells.

The toxicity of the PILs towards the HaCat dermal cells may be affected by the physicochemical properties of the PILs, in addition to their chemical structures. Consequently, the physicochemical properties of viscosity, surface tension and density were obtained for each of these aqueous PIL solutions between 0.75 and 20 mol% of the PILs, and are provided in Figs. 7–9 respectively.

The surface tension for all the PIL-water solutions decreased on addition of the PIL, and the relative order was very similar for all PIL concentrations, as shown in Fig. 7. Similarly, the relative order of the values was consistent with values previously reported for the corresponding neat PILs [38]. The highest and lowest surface tensions were observed for the solutions containing EtAF and EAF respectively. The similarity in the toxicity for these two PILs, as shown in Fig. 5, indicates that the surface tension does not have a significant effect on the toxicities. While, the PILs with the hydroxyl group on the cation had higher surface tensions and lower toxicities, this is perhaps a feature of the hydroxyl group leading to both these effects, rather than the surface tension affecting the toxicity. The density is provided in Fig. 8 and had no correlation with the toxicity of the PILs towards the HaCat cells.

The viscosity of the aqueous PIL solutions had a large variation depending on which PIL, and the PIL concentration, which can be seen in Fig. 9. In general, the PILs that were more viscous were less toxic, which may be due to their transport properties with slower diffusion rates for higher viscosities. Comparing Fig. 9 and Table 1 it is evident that the acetates and glycolates both had the lowest toxicities and highest viscosities, followed by the formates and then the nitrates. However, we note that again this could be correlation and not causation, and further experiments would be required to determine if this is the case. A beneficial effect of viscosity is plausible, since it would lead to a slower change in the osmotic pressure inside and outside the cell line, thus causing less disruption to the cell membrane.

4. Discussion

The PILs used in this investigation share many similarities with conventional salts when used as aqueous solutions. However, the PILs have a significant difference in that all of the ones used here are miscible with water, and hence there is no solubility limit. It is important to note that the toxicity of ILs, PILs and molecular solvents may vary considerably depending on the cell line. The biodegradability of PILs varies depending on which PIL, and which investigation, with reports of them having low biodegradability up to being potentially biodegradable [15–17,39].

The mechanism of cell death can be broadly classified as apoptosis or necrosis, where apoptosis is programmed cell death regulated due to

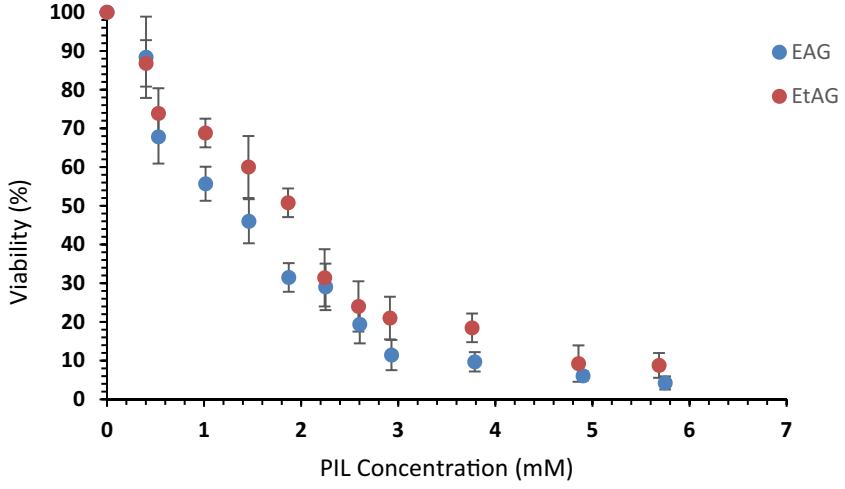

Fig. 4. Viability of HaCat cells towards EAG and EtAG.

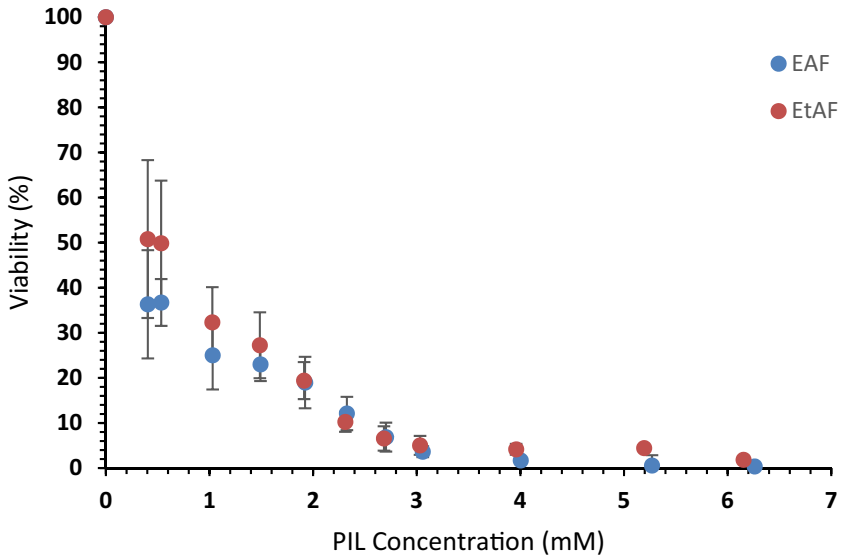

Fig. 5. Viability of HaCat cells towards EAF and EtAF.

specific cellular signals, whereas necrosis is a form of cell injury which results in the premature death of the cells due to external factors. There is limited information about the understanding of toxicity mechanism of ILs towards specific cells [24,40–42], though it is likely that the ILs cause serious damage to the cells, leading to self-destruction (apoptosis) which is due to the IL invading the cell membrane leading to cell death [43]. The mechanism of IL toxicity differs somewhat depending on the type of IL, IL concentration, and cell type, but there is evidence suggesting that the anion has the larger contribution to the IL toxicity [11,26,44,45], which is consistent with what we observed, with the toxicity following nitrates > formates > acetates and glycolates. We anticipate that the mechanism will be similar to salts, with the osmotic pressure caused by the PILs leading to the cells shrinking and hence destabilising the membrane. When we compare the PILs and salts used in this study then it is evident that for the HaCat cell line many of the PILs are significantly less toxic than the potassium nitrate or sodium acetate. This suggests that the acetate and glycolate PILs are having less effect on the cell membrane than the nitrate and formate PILs and less than many conventional salts at equivalent concentrations.

A recent study by Zanoni et al. investigated the toxicity towards HaCat cells of a selection of 13 PILs, which included EtAA, DEtAA and EtAF that were also used in this study [46]. Similar to this study, they also observed that EtAA was less toxic than DEtAA, though their findings were that EtAA and EtAF had comparable toxicities towards HaCat cells [46]. However, there was insufficient experimental detail or data provided to enable a rigorous comparison. The values in this study were approximately an order of magnitude larger, which we tentatively suggest may be due to differences in the washing protocols. In our study we deliberately did not wash with PBS before adding the MTT, due to concern that any cells which were in the process of dying may only be loosely attached, and hence washed away, which would lead to lower EC_{50} values. Interestingly, both studies have shown that the acetate and formate anions lead to relatively low toxicities compared to other commonly used anions for PILs. In their study they attribute this to the

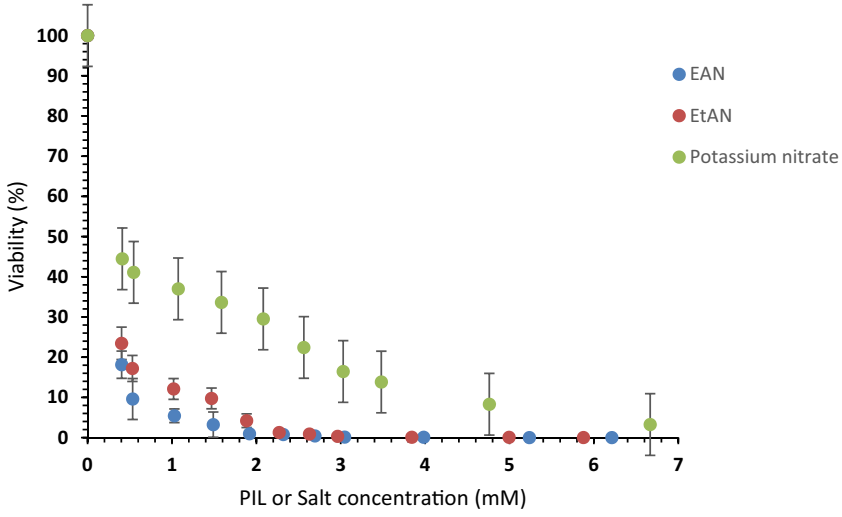

Fig. 6. Viability of HaCat cells towards EAN, EtAN and potassium nitrate.

Table 1
EC_{50} Values for each solvent or salt towards the HaCat cells.

Solvent and salt additive	EC_{50} (±0.5) mM
Ethylammonium formate (EAF)	<0.4
Ethanolammonium formate (EtAF)	~0.4
Ethylammonium glycolate (EAG)	1.3
Ethanolammonium glycolate (EtAG)	1.7
Ethylammonium acetate (EAA)	2.4
Ethanolammmonium acetate (EtAA)	2.5
Diethanolammonium acetate (DEtAA)	0.9
Triethanolammonium acetate (TEtAA)	1.6
Ethylammonium nitrate (EAN)	<0.4
Ethanolammonium nitrate (EtAN)	<0.4
Choline chloride ($ChCl_2$)	3.2
DMSO	2.8
Potassium nitrate	<0.4
Sodium acetate	1.4
Ethanol	<0.4

absence of an alkyl chain, where longer alkyl chains can disrupt the lipid bilayer [46].

In this study, choline chloride was the least toxic of the compounds tested towards the HaCat cells, with an EC_{50} of 3.2 mM. However, EtAA and EAA had EC_{50} values of 2.5 mM and 2.4 mM, respectively, which were relatively similar, and show that as a solvent class, PILs have potential to be tailored to have lower toxicity than many ILs, and solvents such as DMSO. Hence, they are potentially useful solvent additives for cell applications which require a modified solvent environment. Typically, solvent additives would be present at lower concentrations, and when we compare the toxicities of the PILs, salts and solvents at 1 mM, then EAA, EtAA, ChCl, TEtAA have higher, or comparable cell viabilities, to DMSO.

One of the highest toxicities in this study was due to ethanol. This indicates that the toxic behaviour of the PILs is not due to their amphiphilic nature. In addition, along with choline salts, PILs are considered among the most biodegradable ILs reported [5]. Plant based toxicity studies on terrestrial ecotoxicity using short aliphatic PILs such as ethanolamine, diethanolamine and triethanolamine paired with formic, acetic, propionic and pentanoic acids from varying concentrations of PILs at 1, 10, 100, 1000 and 5000 mg/kg dry soil showed nontoxic effect on plants and was biodegradable in soil. The terrestrial plant test in the aqueous solutions of PILs added to growing seedlings of different plants soil microorganisms' carbon transformation test and nitrogen transformation test [15].

While DMSO is a widely used solvent in cell studies, it is typically used at concentrations at or below 1% for cell viability studies. However, when used for cryoprotection it is typically used closer to 10%, and while it has detrimental effects due to its toxicity at these much higher concentration, some degree of toxicity is acceptable due to a lack of other cryoprotectants for some cells. This work highlights that PILs have the potential to be designed to have acceptable toxicities for development as cryoprotectants.

In our previous work we investigated the secondary structural changes of proteins and their stability using FTIR spectroscopy. Specifically, the secondary structure of the proteins of lysozyme, trypsin, α-amylase and β-lactoglobulin were explored in the same 10 PILs used in this study. Interestingly, there are vastly different results seen between the protein stability for different proteins in the PILs, and between that previous work and the toxicity of these cells in the same PIL solutions. It was noted that all four proteins generally retained their secondary structure in EAN and EtAF, whereas in this study we identified EAN as the most toxic, and EtAF as one of the more toxic PILs towards the HaCat cells. All four proteins were insoluble in aqueous solutions of 5 to 50 mol% of EAA, EtAA or DEtAA, whereas lysozyme, α-amylase and β-lactoglobulin were soluble in aqueous solutions of 5–20 mol% of TEtAA [47]. This correlates well with the toxicity data, where we propose the acetates are modifying the solvent interactions such that there are unfavourable interactions, and hence weak IL-protein interactions. Tentatively we suggest that a similar mechanism is present for the cells, with weak interactions between the cells and the acetate ions leading to low toxicities. Larger systematic studies are likely to be necessary to enable any structure-property relationships to be identified which can be used across multiple proteins or cells. In addition, the acetate based PILs are highly viscous compared to the other ILs used and they showed a lower toxic effect on the cells which suggests that higher viscosities may decrease the IL toxicity towards these cells, perhaps through slower diffusion rates over these timescales.

Even though the surface tension and density did not appear to have a direct effect on the toxicity, it is important to understand these physiochemical properties for some applications. The effect of viscosity on toxicity, along with the changing cation/anion structure on toxicity is consistent with previous studies, which reported that [EMIM]Cl and [BMIM]Cl which are imidazolium based ILs with higher viscosities, showed a lower toxic effect on PC3 human prostate cancer cells, which were used as a standard model system which have robust and reproducible cell lines [48]. More broadly, previous studies have shown that the toxicity of aprotic ILs is dependent on their interaction with cellular membranes [49–51] and is affected by the IL alkyl chain length, cation and anion combination, along with the structure or morphology of the organism, or the cells used [52–55].

Overall this study highlights the large variety of properties which can be observed from PILs with relatively similar chemical structures. The toxicity of EtAA and EAA was higher than choline chloride, but

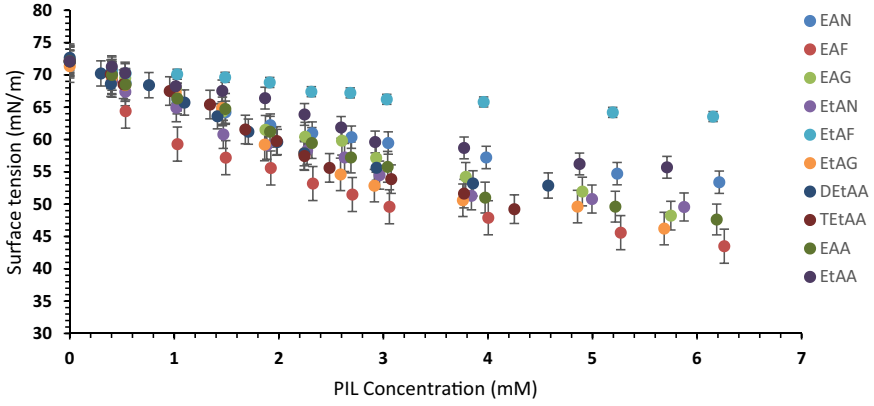

Fig. 7. The surface tension of the PILs (mN/m) was calculated in the temperature range (22.0 ±0.5) uncertainty for all the PILs expect for formates with (±20).

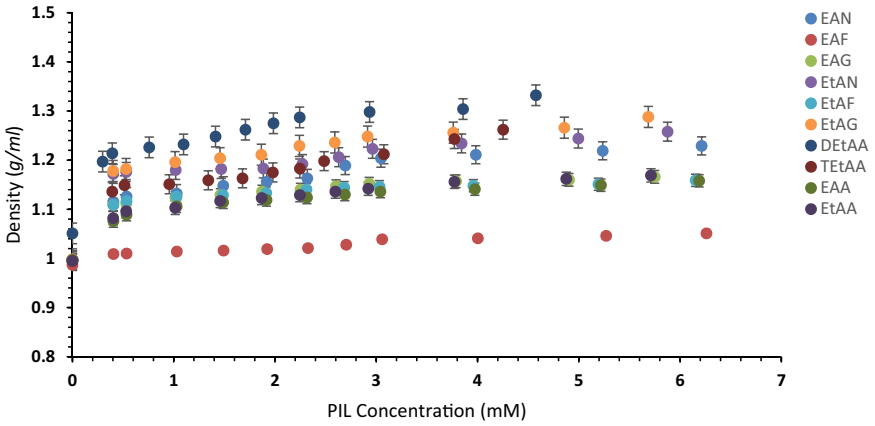

Fig. 8. Densities (ρ) of the PILs were measured with ($\pm$0.5) uncertainty for all the PILs.

sufficiently similar to suggest that some PILs can be developed to be among the most biocompatible ILs. There are numerous applications with proteins and cells which require solvent modification while maintaining protein structure/activity and cell viability. The potential low toxicity, low cost and simplicity of design and synthesis of PILs has them well placed for development for these applications.

5. Conclusion

This study shows that PILs are viable solvent additives for biological media and can be designed/selected to have comparable toxicities to the commonly used DMSO. The comparison of the PILs, molecular solvents and salts indicate that changes in the EC_{50} values are not a salt effect, or purely due to amphiphilic properties. It was observed that the PIL anion had a more dominant effect than the cation on toxicity, with the acetate containing PILs the least toxic, and the nitrate PILs the most toxic. Ethanolammonium acetate and ethylammonium acetate had the highest EC_{50} values of the PILs, and were only slightly more toxic than choline chloride and DMSO. This indicates that some PILs are viable solvent additives for use with cells where the solvent medium needs modifying. It is also evident from this study that some PILs are very toxic towards HaCat cells, such as the nitrate containing PILs. Consistent with other studies, higher viscosities correlated to lower toxicities. Further experiments are needed to assess the general toxicity of this class of commonly used PILs towards a broader range of cell types.

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CRediT authorship contribution statement

Radhika Arunkumar: Investigation, Writing - original draft. **Amanda N. Abraham:** Methodology, Resources. **Ravi Shukla:** Conceptualization, Resources. **Calum J. Drummond:** Supervision. **Tamar L. Greaves:** Conceptualization, Writing - review & editing.

Declaration of competing interest

The authors state that there are no financial or personal interests or beliefs which are affecting our objectivity for this paper.

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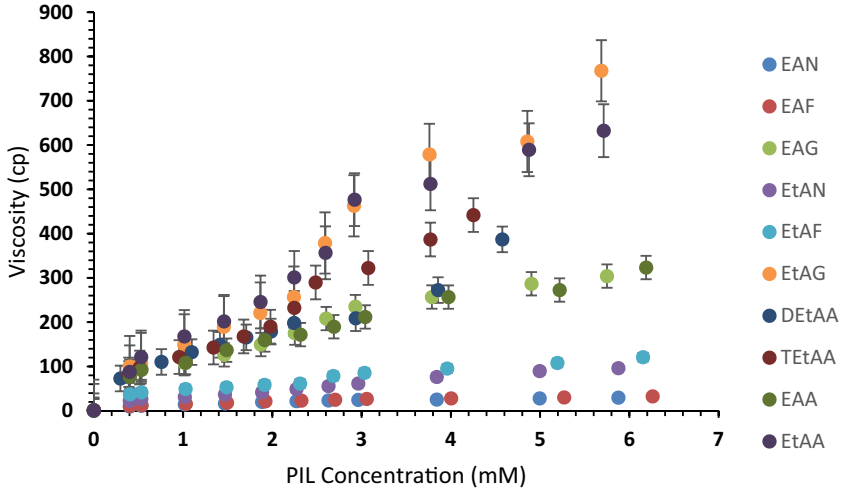

Fig. 9. Viscosity (η) of the PILs were measured with ($\pm$0.5) uncertainty for all the PILs.

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