

# Understanding the physical properties, toxicities and anti-microbial activities of choline-amino acid-based salts: low-toxic variants of ionic liquids

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## Abstract

Ionic liquids (ILs) are often touted as potential ‘green’ substitutes for volatile organic compounds in process chemistry. However, their often high solubility in water limits their applications since discharge of spent ILs into natural water bodies may have significant detrimental eco-toxicological consequences for aquatic organisms. This is especially true for most imidazolium and pyrdinium based ILs which have been proven as toxic to aquatic life

organisms. The challenge is therefore to synthesize ILs which exhibit inherently low aquatic toxicity. In the present work, five choline based ILs pairing five different amino acid anions [Cho][AA] have been synthesized and characterized. Their thermal stability, density, viscosity and thermal expansion coefficient were reported. The toxicity behavior of a synthesized ILs were investigated on guppy fish (*Poecilia reticulata*) and three microbial species. Fish acute toxicity test reveal that the studied ILs could be classified as non-toxic while their 50% effective concentrations (EC<sub>50</sub>) were found to be comparable to the least toxic imidazolium-based ILs.

**Keywords:** Amino acid; ionic liquids; choline; toxicity; guppy fish; human pathogens bacteria

## 1. Introduction

Room temperature ionic liquids (RTILs) are mostly salts that bear an organic cation, obtained by the extension of a valence of a nitrogen, phosphorus or sulphur atom and an organic or inorganic anion with melting points below or not too far above ambient temperatures. They are touted as potential ‘green’ substitutes for volatile organic compounds in process chemistry and the food industry [1]. The discovery of amino acid-based ionic liquids (AAILs) has generated widespread interest due to their favorable characteristics which include good task-specificity and relatively low toxicity. Amino acids are essentially bio-renewable and non-toxic chiral compounds which have found applications as organocatalysts [2].

Natural amino acids were first used in 2005 by Fukumoto and co-researchers [3] for the synthesis of 1-ethyl-3-methylimidazolium-based ILs. They also discovered that the miscibility of ILs with organic solvents was dependent upon the side-chain structure of the

corresponding amino acid anion. Thus, in various fields, such as industrial chemistry and pharmaceutical chemistry, these AAILs could be used for various applications such as intermediates for peptide syntheses [4] and chiral solvents [5]. Abbott and co-researchers [6] proposed a simple approach to prepare ILs based on quaternary ammonium salt such as choline (2-hydroxyethyl-trimethylammonium) which was a water-soluble essential nutrient. Choline chloride has a melting point up to 302°C and when mixed with metals salts such as tin(II) chloride and zinc(II) chloride it can be transformed into so-called “deep eutectic solvents”. In 2007, Hu and co-researchers [7] investigated the use of choline-based ILs to prepare novel catalysts for organic synthesis. In their work, the common aminoacid L-Proline was associated with choline and the investigated ILs were able to catalyze direct aldol reactions with good yields and short-time reaction. N-methyl-D-glucamine (NMDG), also known as Meglumine, is a derivative of sorbitol that contains amethylamino group. Research fields involving NMDG are essentially metal removal by polyamine composites due to its chelating properties [8]. In 2012, Joshi and co-researchers [9] managed to synthesize glucaminium-based ILs for the removal of boron from water.

Despite the fact that ILs have been widely studied and applied for various industrial processes, the (eco)toxicological risk profile of ILs is still scarce and, in some cases, non-existent. In general, there are several ways to establish the toxicities of ILs whereby they can be tested on aquatic species, bacteria and cells. Freshwater algae, daphnia and fish are usually used for the assessment of IL toxicities. Such assessment is imperative because aquatic organisms are the first recipients of most toxic substances generated by industrial, agricultural and domestic activities. Acute toxicity bioassays can be established by exposing the aquatic organisms to different concentrations of the tested substance on 96-hour exposure duration. The concentration that causes 50% of mortality of the organism is then determined and defined as its Lethal Concentration<sub>50</sub> (LC<sub>50</sub>). One of the earliest approaches on

determining fish toxicity has been conducted for 15 ammonium cation-bearing ILs by Prettiand co-researchers using zebra fish [10]. In their study, 13 out of 15 ILs had LC<sub>50</sub> values greater than 100ppm which could be regarded as “non-highly lethal” towards that species.

Bacteria perform many critical roles in ecosystem function and productivity. The antimicrobial activity is related to a specific effect of the substance (e.g., reproduction, growth) and expressed as EC<sub>50</sub> (half maximal effective concentration) which is defined as effective concentration of IL required for 50 % toxicity within a specified exposure time. Most of the studies of the ILs effect on microorganisms had been conducted on a marine bacteria called *Vibrio Fischeri* [11, 12] which indicated that the inhibition of luminescence increased with increasing n-alkyl chain length but no systematic influence of the anion could be determined. Other types of bacteria have also been studied as well. Pernakand co-researchers [13] conducted bioassays using the same species with imidazolium cation combined with lactate anion. They showed that antimicrobial activity is related to the length of the lactate substituent, short substituents being inactive.

In the present study, the synthesis and characterization of ILs using choline-amino acid-based precursors [Che][AA] have been conducted. Amino acids have been chosen to assume the role of anion, while choline was selected because cholinium cation is the basis of a bioactive family of ILs. These precursors have been touted as ‘natural’ which are considered (and postulated) by many as possessing inherently low toxicity. However, their toxicological profiles are largely not established due to the lack of toxicology assessment. As such, this study aims to provide a comprehensive understanding of the toxicities and anti-microbial activities of synthesized [Cho][AA] ILs. Primary toxicity data profiles have been developed for two trophic level organisms such as guppy fish and human pathogenic bacteria because dose-response bioassays on test organisms are required by EU regulations (Dir. 67/548, 88/379, and 76/769; Reg. 793/93) and the new EU regulatory framework REACH.

## 2. Materials and Methods

### 2.1. Synthesis of ILs

The route to prepare choline-amino acids-based ILs, [Cho][AA] is similar to a previous study which synthesized imidazolium-based ILs with amino-acids [3, 14, 15] and optimized by Hu and co-researchers [7]. An aqueous solution of choline hydroxide (Sigma-Aldrich, 20wt% in water) was added to excess equimolar amino acids (all from Sigma Aldrich). The mixture was stirred at room temperature for 48hours. Water was then removed at 60°C under vacuum. A light-yellow oily ILs was obtained as well as a white precipitate which corresponds to unreacted amino acid. A mixture of acetonitrile and methanol (7:3) was used to wash the final product and separate the unreacted amino acids. Subsequently, the mixture was filtered and the filtrate was then evaporated to remove the solvent. The product was finally dried under vacuum for 4 days at 60°C.

### 2.2. Characterization of ILs

The infrared spectra were obtained from Fourier-Transform Infrared Spectrometer (FT-IR) equipped with single-reflection ATR (Attenuated Total Reflectance) accessory. The samples were dripped onto the ATR crystal/prism (zinc selenide) and run for the spectrum. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker 400 MHz (Avance, Billerica, USA) to establish the ILs structures. Chemical shift,  $\delta$ , is reported in ppm relative to the tetramethylsilane (TMS) which was used as an internal standard. Deuterated solvents such as D<sub>2</sub>O and DMSO were used. The sample (5 mg) was dissolved in 0.7 cm<sup>3</sup> of the deuterated solvent. The water content of the synthesized ILs was determined using a coulometric Karl Fischer titrator (Mettler Toledo DL 39) with a CombiCoulomat Karl Fischer reagent (Merck) using hydranal as the titrating reagent. The samples were injected into the titration cell and the weight value was entered in the titrator for analysis. Measurements were performed in

triplicate immediately after drying under vacuum. The average values obtained for the ILs must be below 1000 ppm to be acceptable.

### 2.3. Thermophysical studies

The density and viscosity of the ILs were determined using a SVM 3000 Viscometer<sup>TM</sup> (Anton Paar). Density was measured at temperatures from 25 to 70°C and the values were recorded every 5°C. IL samples (5 mL) were slowly injected in a U-tube using a syringe. Thermal stability of the studied ILs was measured using a PerkinElmer Pyris V-3.81 thermal gravimetric analyzer. Sealed aluminum pans were filled with the ILs samples and heated at rate of 10°C.min<sup>-1</sup> under Nitrogen atmosphere.

### 2.4. Toxicity analyses

The fish toxicity tests were conducted according to methods approved by standard organizations such as Environmental Protection Agency (EPA) [16] and Organization for Economic Co-operation and Development (OECD) [17]. Guppy fish (*Poecilia reticulata*) was selected for this study because it is one of the most common fish used for laboratory toxicity studies and its one of the tested organisms which recommended by OECD. The first step of assessment is the collection of fish and their culture. Guppy fish (*Poecilia reticulata*) obtained from a fish hatchery in Perak, Malaysia was chosen to undergo the toxicity analysis. The fish were screened based on their sizes ( $\pm 2$ cm). They were then placed in plastic tanks filled with 5L water provided with one electric air pump (1.8L/min). The air flows from the pump into the tank through a plastic tube connected with air-filter to its end. Specific subsistence conditions were adhered to ensure optimum health of the fish (see [Table S1](#)).

Initial acute toxicity assessment was performed based on 100 mg/L as limited test. According to OECD guidelines [17], the absence of mortality indicates that the fish is not the

most sensitive species. However, a choice was made to conduct the full assay for ILs even though their LC<sub>50</sub> values fall above 100 mg.L<sup>-1</sup> to establish the exact hazard ranking. The obtained LC<sub>50</sub> values were then compared with a hazard ranking developed by Passino and Smith [18] listed in Table 1.

Table 1: Hazard ranking developed by Passino and Smith [18]

Relative toxicity	Concentration (mg/L)	Hazard Ranking
Highly toxic	0.1-1	+++++
Moderately toxic	1-10	++++
Slightly toxic	10-100	+++
Practically harmless	100-1000	++
Relatively harmless	>1000	+

### 2.5. Anti-microbial activity of ILs

The ILs were assayed for anti-microbial activity against three registered microbial isolates, namely, *Listeria monocytogenes*, *Aeromonashydrophila* and *Klebsiella pneumonia*. Determination of the EC<sub>50</sub> was conducted using the broth tube dilution method [19]. The bacteria strains were cultured on a Muller-Hinton broth (MHB) for 24 hours. Then, to each well of 96-well plates, 100µL of MHB medium was injected and inoculated with 100µL of bacterial suspension. The tested ILs (100µL) were then dissolved in MHB and added to the first two rows in the 96-well plates and serial two-fold dilutions were made from the second row and continued up to the seventh rows. The last row was kept untreated as a control in order to produce a pure colony. Three replicates and seven different concentrations were studied for each IL. Absorbance reading was taken by a microplate reader (Thermo Scientific Multiskan FC) at wavelength 620nm and EC<sub>50</sub> was determined by a graded dose response curve and represented the concentration of a compound where 50% of its effect was observed.

### 3. Results and Discussion

#### 3.1. Chemical structures of ILs

Figure 1 shows the chemical structures of all the synthesized choline-amino acid ILs, namely, choline serinate [Cho][Ser], choline valinate [Cho][Val], choline proline [Cho][Pro], choline histidinate [Cho][His] and cholinealaninate [Cho][Ala] ILs. The percentage water contents of the ILs are 0.57, 0.98, 0.93, 0.74 and 0.77wt.%, respectively. The IR spectra for [Cho][AA] ILs confirm the presence of choline and the two amino acids (Figure 2). The amine functions of L-Proline and L-Alanine are observed at 3178 and 3128  $\text{cm}^{-1}$ , and, 3032 and 3136  $\text{cm}^{-1}$ , respectively, in which the latter is overlapped by the free O-H stretch of water and choline. A few peaks are observable on the left of these values and can be attributed to the existence of primary or secondary amine salts. The peaks at  $\sim 1738 \text{ cm}^{-1}$  are attributed to the N-H bending for both amino acids. The strong bands at 1582  $\text{cm}^{-1}$  for L-Proline and 1563  $\text{cm}^{-1}$  for L-Alanine correspond to the asymmetric stretch of the carboxylate function. Indeed, the frequency of C=O absorption is lowered from the value found for the parent carboxylic function because of resonance. As for the symmetric stretch of this function, this appears at  $\sim 1400 \text{ cm}^{-1}$ . On the spectra of both [Cho][Pro] and [Cho][Ala], characteristic peaks of the cholinium structure are observed. The broad band at  $\sim 3200 \text{ cm}^{-1}$  is assigned for O-H stretching vibration, the peak near 1635  $\text{cm}^{-1}$  on the choline hydroxide spectrum is observed at roughly 1738  $\text{cm}^{-1}$  for the two amino acid ILs and attributable to the bending of quaternary ammonium group, the peak at  $\sim 2900 \text{ cm}^{-1}$  is due to the stretching vibration band from C-H, the peak at 1475  $\text{cm}^{-1}$  is the C-H of  $-\text{CH}_3$  stretching vibration band due to the methyl groups of ammonium, at  $\sim 1360 \text{ cm}^{-1}$  the C-N stretching vibration is observed and the peak at  $\sim 1085 \text{ cm}^{-1}$  is due to the C-O stretch of primary alcohol.  $^1\text{H}$  NMR analysis shows the characteristic signals of choline and amino acids. For example, the following signals of choline are observed for [Cho][Val] (Figure S1):  $\delta_{\text{H}}$  3.16 (9H, s,  $\text{CH}_3$ ),



3.48 (2H, t, CH<sub>2</sub>, J=6.62 Hz), 4.01 (2H, t, CH<sub>2</sub>, J=6.61 Hz) ppm. The valinate anion provides these signals: 0.82 (3H, d, J=6.78 Hz), 0.89 (3H, d, J=6.79 Hz), 1.88 (1H, heptd, J=6.79, J=6.18 Hz), 3.01 (1H, d, J=6.17 Hz) ppm.

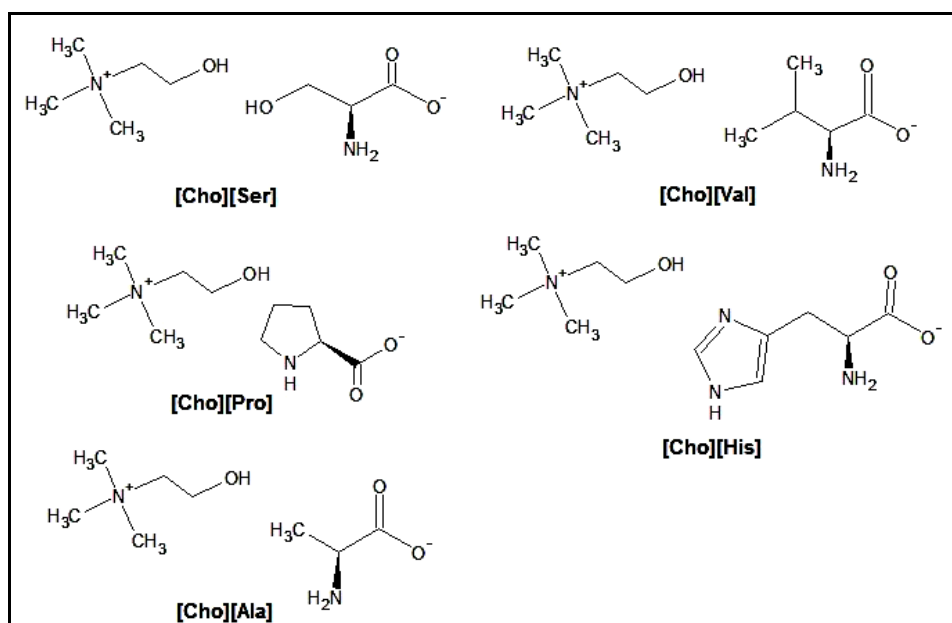


Figure 1: Chemical structures of the synthesized ILs.

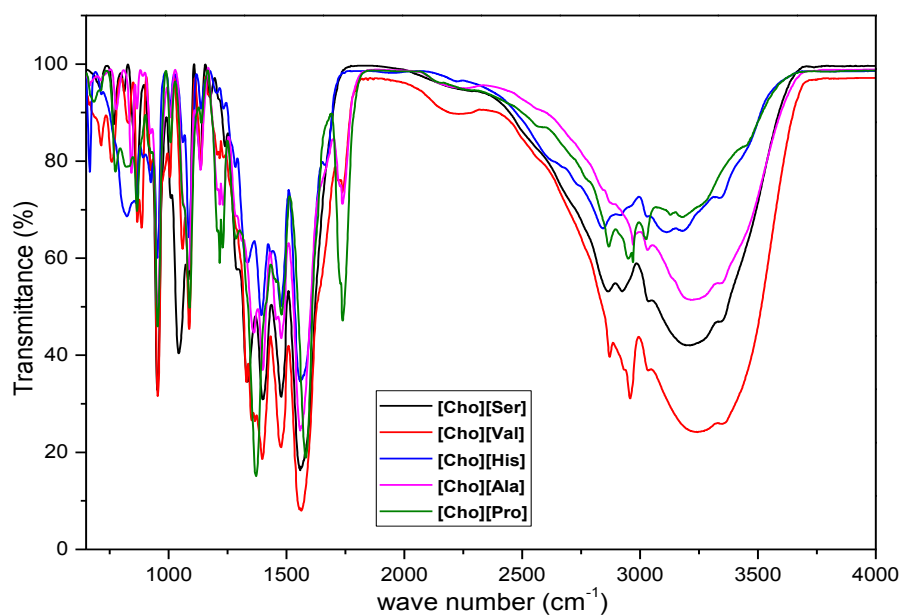


Figure 2: IR spectra for choline-amino acid ILs, namely, [Cho][Pro], [Cho][Ala] and [Cho][OH].

### 3.2. Thermophysical studies of the ILs

The densities of the ILs are plotted as a function of temperature (Figure 3) and the data was tabulated in Table S2. [Cho][Ala] IL possesses the lowest density while [Cho][Ser] IL has the distinction of having the highest density. Over the present temperature range, the densities of all five ILs decrease linearly with increasing temperature in which there appears to be a correlation of density with amino acid anion. This phenomenon can be explained by steric effects. The steric hindrance of serine is higher than alanine because of the hydroxyl group on position  $\beta$  of the amine group. The second highest density is attributed to [Cho][His] IL and is justified by the presence of the aromatic ring on position  $\beta$ . By comparing the structures of the different amino acids used, it becomes apparent that the densities of such ILs tend to increase with the steric hindrance of the anion structure. The experimental densities ( $\rho$ ) were fitted using the least-square method based on Eq. 1:

$$\rho(g.cm^{-3}) = A_0 + A_1T \quad (1)$$

where  $\rho$  denotes the density of the studied ILs,  $A_0$  and  $A_1$  are the correlation coefficients and  $T$  is the temperature in Kelvin. The correlation coefficients were estimated by least-square fitting method using Equation 1. The estimated values of the fitting parameters and the correlation coefficients were presented in Table 2.

The viscosity values are plotted as a function of temperature (Figure 4). By and large, the viscosity trend is similar to that of density. Indeed, the highest viscosity measured at 25°C is for [Cho][Ser] while the lowest is for [Cho][Ala]. The viscosity decreases exponentially by increasing the temperature. The order from the highest viscosity to the lowest is as follows: [Cho][Ser] > [Cho][His] > [Cho][Val] > [Cho][Pro] > [Cho][Ala].

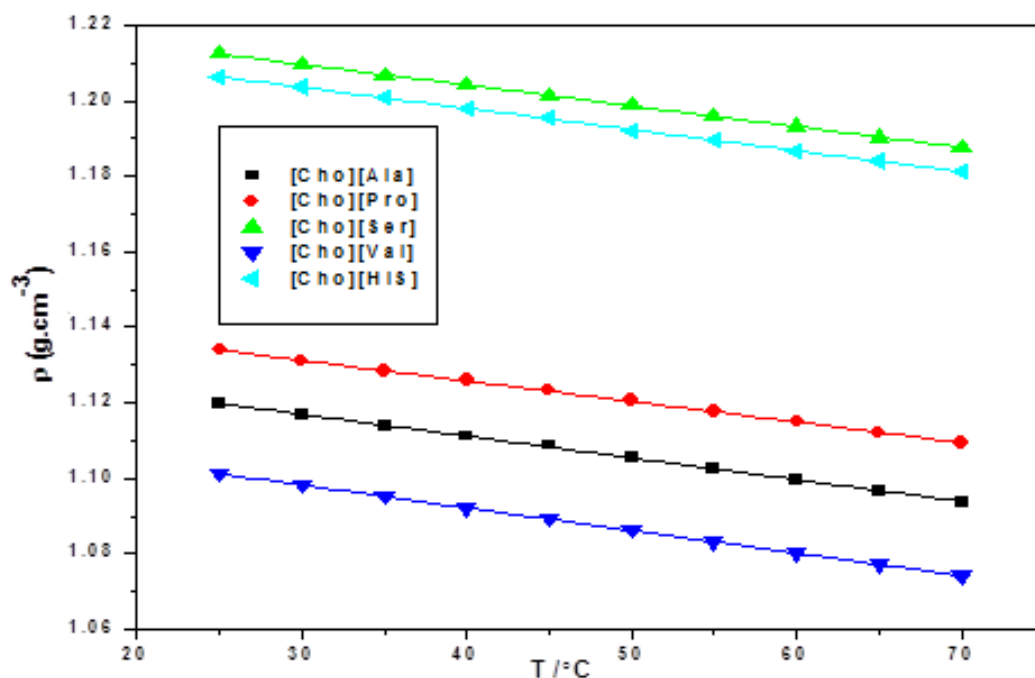


Figure 3: Densities of the synthesized choline-amino acid ILs.

The viscosity of the choline based ILs decreased markedly with increasing temperature. The experimental viscosities ( $\eta$ ) were fitted using Eq.2:

$$\log \eta \text{ mPa.s} = A_2 + \frac{A_3}{T} \quad (2)$$

where  $\eta$  denotes the viscosity of the [Cho][AA], and  $A_2$ ,  $A_3$  are the fitting parameters and  $T$  is the temperature in Kelvin. The values of the fitting parameters for viscosity, together with the correlation coefficients, are presented in Table 2.

The TGA analyses indicate that the  $T_{\text{onset}}$  for the synthesized [Cho][Ser], [Cho][Val], [Cho][Pro], [Cho][His] and [Cho][Ala] ILs were 191, 187, 174, 184 and 180°C, respectively.

It appears that the influences of different anions on the decomposition temperature are marginal. The thermal decomposition temperature of the [Cho][AA] ILs were lower than their imidazolium homogeneous even with the shorter alkyl chain which normally less stable than their counterparts with longer alkyl chain. For example, the decomposition temperature

for 1-ethyl-3-methylimidazolium pairing alanine, serine and proline were 231°C, 239°C and 258°C, respectively [20].

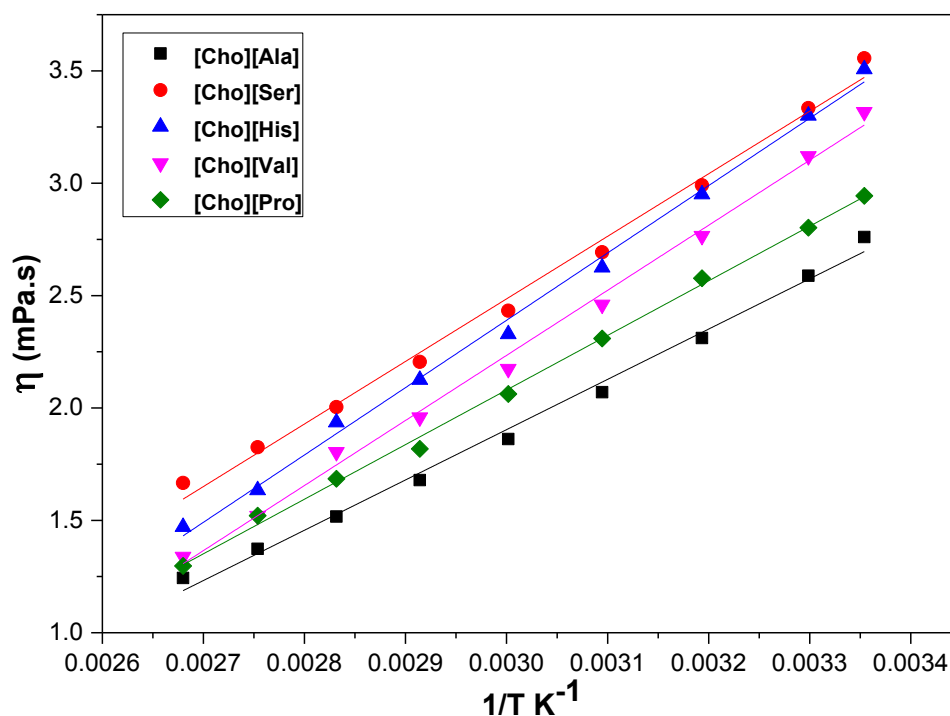


Figure 4: Viscosities of the synthesized choline-amino acid ILs.

Table 2. Fitting parameters and correlation coefficients for the density and viscosity equations.

	[Cho][Ala]	[Cho][Pro]	[Cho][Ser]	[Cho][Val]	[Cho][HIS]
$\rho$					
A	1.13418	1.14761	1.22622	1.1162	1.22038
B	-5.75E-04	-5.44E-04	-5.51E-04	-6.00E-04	-5.59E-04
R <sup>2</sup>	0.99941	0.99969	0.99966	1	0.99936
$\eta$					
C	-4.8095	-5.8650	-6.5988	-6.4517	-5.2082
D	2237.6918	2783.5067	2996.3424	2895.2479	2429.4095
R <sup>2</sup>	0.99394	0.99364	0.99636	0.99582	0.99784

The experimental density values were used to calculate thermal expansion coefficients using Eq. 3:

$$\alpha(K^{-1}) = \frac{1}{V_m} \left( \frac{\partial V_m}{\partial T} \right)_P = -\frac{1}{\rho} \left( \frac{\partial \rho}{\partial T} \right)_P \quad (3)$$

The values of the isobaric thermal expansion coefficients are shown in Table S2.

It can be observed from Table S2 that the coefficients of thermal expansion of choline based ILs do not change appreciably with respect to temperature and show their independence of temperature. The values obtained for above synthesized ILs were also found similar to those reported for imidazolium, pyridinium, phosphonium, and ammonium-based ILs, ( $5.0 \times 10^{-4}$  to  $6.5 \times 10^{-4}$ )  $K^{-1}$  [20].

### 3.3. Acute toxicity of IL

All ILs were initially tested for 100 mg/L. Results show that all tested ILs had 96 h LC<sub>50</sub> values greater than 100 mg/L (Table S3). This indicated that the ILs would not be classified as ‘toxic’ to the fish and therefore the full acute toxicity test could be waived. However, to confirm this finding, the full test was performed for assessing the toxicity of a choline prolinat on a localized guppy fish species, *Poecilia reticulata*. Five concentrations were determined as follows: 700, 750, 775, 800 and 850 ppm. Each tank containing eight fish received one calculated dose of ILs and was inspected after 24, 48, 72 and 96 hours to determine the number of mortality. The following parameters were measured every 24 hours: dissolved oxygen concentration, pH and water temperature. Results show that when choline prolinat was tested on the guppy fish, the latter appear to induce a swollen belly and prolonged tiredness. The observations throughout the toxicity assessment experiment are summarized in Table 3. A representative dose-response (mortality rate) curve of choline prolinat on *Poecilia reticulata* is shown in Figure S2. At 50% of mortality, the half lethal

concentration  $LC_{50}$  can be determined. For [Cho][Pro], the  $LC_{50}$  is indicated to be 800ppm and this is classified as ‘practically harmless’ by the hazard ranking developed by Passino and Smith [18] (see Table 1). At high concentrations, the toxic behavior of this IL might come from the choline cation that decomposes into trimethylamine, a compound toxic to aquatic species [21].

Table 3: Fish toxicity assessment for choline proline

<b>C (ppm)</b>	<b>log C</b>	<b>Number of fish tested</b>	<b>Number of mortality (dead fish) after test</b>	<b>% mortality</b>
700	2.85	8	3	37.5
750	2.88	8	3	37.5
800	2.90	8	4	50.0
850	2.93	8	8	100

### 3.4. Anti-microbial activities of ILs

After screening test was performed, the initial ILs concentration of 1500mM was used for the broth micro dilution test. This concentration was subsequently diluted along the rows of the 96-well plate in order to facilitate investigation on the effect of the ILs’ concentrations on microbial viabilities. A typical example of the viabilities of *Aeromonashydrophila* as a function of [Cho][AA] ILs’ concentrations was shown in Figure 5. Tested ILs were effective against bacteria in a dose-dependent manner since viabilities tend to decrease with higher concentrations. As can be seen from the reported toxicity data, no clear trend can be drawn for the effect of the different amino acid anions on the antimicrobial activity, this behavior is in agreement with several ILs toxicity studies [22, 23].

The antimicrobial activity of 1-(2-hydroxyethyl)imidazolium chloride, 1-(2-hydroxyethyl) -3-methylimidazolium chloride, 1-(2-hydroxyethyl)-3-butylimidazolium chloride and standard antibiotic gentamicin were previously reported against *L.monocytogenes* and *A.hydrophila* [24], the results showed that the 1-(2-hydroxyethyl)-3-

imidazolium chloride based ILs was less potent to *L.monocytogenes* than the choline analogue. Nonetheless, a comparable antimicrobial activity against *A.hydrophila* was observed. Moreover, the reported EC<sub>50</sub> for the choline moiety was lower than the one reported for gentamicin.

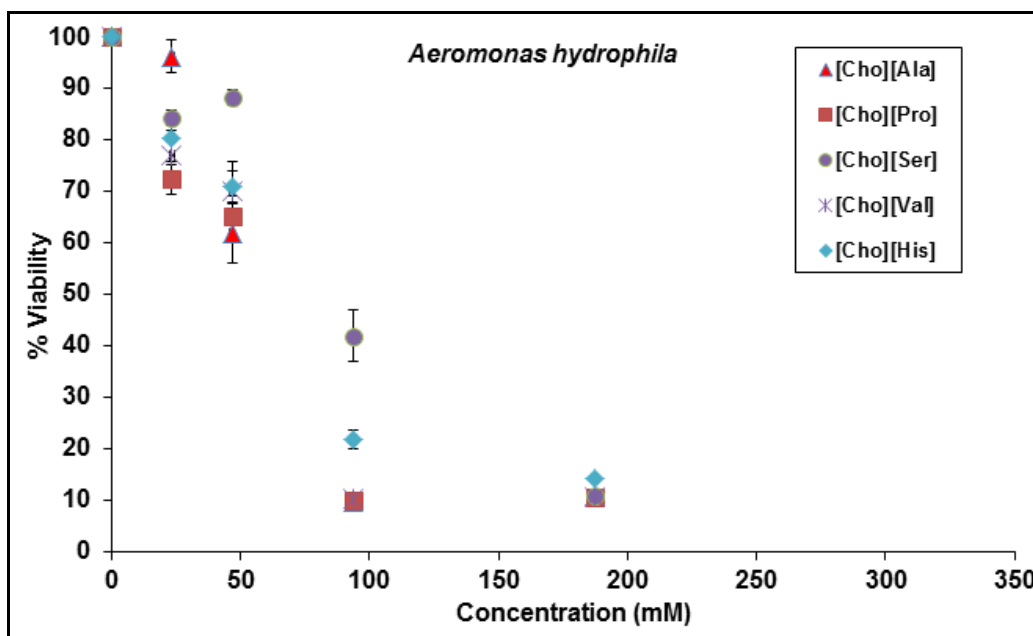


Figure 5: Viabilities of *Aeromonashydrophila* as a function of [Cho][AA] ILs concentrations.

Furthermore, the antimicrobial activity of 1-(2-hydroxyethyl)-3-methylimidazolium [C<sub>2</sub>OHmim] and 1-octyl-3-methylimidazolium [C<sub>8</sub>mim] based amino acid was recently reported against the two similar organisms by Ghanem et al. [25]. The reported data indicated the importance of the cation as antimicrobial potency dominator. Additionally, EC<sub>50</sub> for [C<sub>2</sub>OHmim][Ser], [C<sub>2</sub>OHmim][Pro], [C<sub>2</sub>OHmim][Ala], [C<sub>8</sub>mim][Ser], [C<sub>8</sub>mim][Pro] and [C<sub>8</sub>mim][Ala] against *L. monocytogenes* were found to be 85.46, 48.88, 26.37, 4.68, 3.72 and 2.62 mM respectively. It can be seen that the reported EC<sub>50</sub> for [C<sub>2</sub>OHmim] moiety were comparable or even less than the ones for choline analogue. Also, the EC<sub>50</sub> values clarify that by switching the choline or [C<sub>2</sub>OHmim] cation to their [C<sub>8</sub>mim] counterparts, the EC<sub>50</sub> values were almost decreased ten times and the toxicity is increased. It is an indication that both

choline and [C<sub>2</sub>OHmim] cations exhibit low antimicrobial activity compared to imidazolium cation as it possesses a long alkyl chain (octyl and above).

As evident by the data reported in Table 3, all ILs proved to be relatively toxic toward the microbial species. Practically all of the tested ILs have similar EC<sub>50</sub> values for the same microbial species except [Cho][Ser] IL. Indeed, [Cho][Ser] IL seems to have a lower antimicrobial activity than the other ILs against *Klebsiella pneumoniae* and *Aeromonashydrophila*. This is probably attributed to the fact that L-Serine possesses one more hydroxyl group than the other tested amino acids. The presence of aminoacetate anions does not seem to affect the toxicities of the ILs taking into account their EC<sub>50</sub>. Recent toxicological studies have focused on a new class of ILs with increased biodegradability through the incorporation of oxygen atoms [26]. It was previously shown that the introduction of one oxygen atom into the lateral chain of imidazolium-based ILs seemed to decrease the toxicity of the IL with respect to alkyl counter parts towards the crustacean *Daphnia magna* and the bacterium *Vibrio fischeri* [27, 28]. This observation is in agreement with our observation - the low anti-microbial activity of [Cho][Ser] compared to the other ILs in the present study. Nevertheless, the close values obtained for EC<sub>50</sub> do not seem to confirm the influence of choline or the amino acids on their toxicities. It is interesting to note that some species are more susceptible than others. *Aeromonashydrophila* is less resistant than *Klebsiella pneumoniae* and *Listeria monocytogenes*.



Table 4: Acute toxicity of the synthesized ILs on the three microbial species

ILs	<i>Klebsiella pneumoniae</i>			<i>Listeria monocytogenes</i>		<i>Aeromonashydrophila</i>	
	Mwt	EC <sub>50</sub> (mM)	EC <sub>50</sub> gmole.L <sup>-1</sup>	EC <sub>50</sub> (mM)	EC <sub>50</sub> gmole.L <sup>-1</sup>	EC <sub>50</sub> (mM)	EC <sub>50</sub> gmole.L <sup>-1</sup>
[Cho][Ser]	208.26	135.78±0.07	28279.55	34.34±0.09	7151.65	43.11±0.07	8978.09
[Cho][Val]	220.31	64.32±0.06	14170.89	38.14±0.08	8402.62	27.13±0.08	5977.01
[Cho][Pro]	218.29	64.14±0.06	14001.76	36.67±0.10	8004.69	24.75±0.10	5843.62
[Cho][Ala]	192.26	63.08±0.06	12128.26	42.67±0.09	8203.73	26.77±0.06	5146.80
[Cho][His]	258.32	68.5±0.07	17694.92	39.5±0.08	10203.64	30.85±0.08	7969.17

#### 4. Conclusions

Choline serinate, choline valinate, choline proline, choline histidinate and choline alaninate ILs were synthesized and characterized, and their toxicities and anti-microbial activities established. The toxicological study tested on local guppy fish and anti-microbial investigations implied that these choline-amino acid ILs could be classified as non-toxic, albeit their anti-microbial potency may be lacking to fully function as an antibiotic. Our investigation suggests that the use of these new ILs as solvents may be favorable due to its confirmed relative low toxicity, though careful discretion must obviously be exercised when used. The findings from this study can be used for better design of choline-amino acid-based ILs with consideration of their aquatic toxicities.

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