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Acute Toxicitystudy of Choline Based Ionic Liquids Towards *Danio rerio* fish and the Aggregation Behavior of Their Binary Mixtures

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Abstract

Marine oil spills are effectively controlled by chemical dispersants. However, the toxicity associated with it reduce its employment in marine environment. To overcome this limitation, the acute toxicity of choline based ionic liquids was evaluated as a potential low toxic variant for oil spill remediation. Further, the aggregation behavior of the individual as well as their binary mixtures was also evaluated by employing tensiometry technique. The half-lethal concentration, LC_{50}on zebrafish (*Danio rerio*) of three choline based ionic liquids showed that the studied ionic liquids (ILs) fall in the range of "practically nontoxic" (100-1000 mg L^{-1}).Various micellar properties showed that a synergistic interaction existed between all the binary mixtures ($\beta < 0$, $f_1$and f_2 <1). Moreover, the produced micelles were found to be spontaneous and thermodynamically stable with respect to all the mole ratio of ILs. Overall, these results showed the safe nature of the studied ILs for various application including oil dispersants.

Abstract

Uji Toksisitas Akut dari Ionic Liquids (IL) Berbasis Kolin terhadap Ikan *Danio rerio* dan Perilaku Agresi dari Campuran Binernya. Tumpahan minyak bumi di laut diatasi secara efektif dengan dispersan yang berasal dari bahan kimia. Namun, karena toksisitas yang ditimbulkan oleh dispersan tersebut, penggunaan dispersan tersebut dikurangi dalam lingkungan laut. Untuk mengatasi keterbatasan ini, uji toksisitas akut dari *ionic liquid* (IL) berbasis kolin dievaluasi sebagai potensial toksik varian rendah untuk remediasi tumpahan minyak bumi. Selanjutnya, perilaku agregasi individu serta cempuran binernya juga dievaluasi menggunakan teknik tensiometri. LC50 dari *zebrafish* (*Danio rerio*) untuk tiga IL berbasis kolin menunjukkan bahwa cairan yang diteliti berada dalam kisaran "tidak beracun" (100–1000 mgL^{-1}). Sifat-sifat *micellar* menunjukkan bahwa interaksi sinergis terdapat pada semua campuran biner ($\beta < 0$, $f_1$and f_2 <1). Selain itu, *micelle* yang diproduksi dengan semua rasio mol dari IL bersifat spontan dan stabil secara termodinamika. Secara keseluruhan, hasil ini menunjukkan bahwa IL yang diteliti bersifat aman untuk alam dan dapat digunakan dalam berbagai aplikasi termasuk dispersan untuk minyak bumi.

Keywords: dispersant, ionic liquids, oil spill, toxicity

1. Introduction

The transfer of crude oil and its treated products due to various activities adversally effected the marine environment [1]. Therefore, to reduce the impact of oil spill, several response measures were adopted and among them chemical dispersant is provent to be the most suitable response option [2]. Although it is well accepted that employment of chemical dispersants reduce the damages of oils pill but still its use is restricted in marine environment by many governmental organizations due to toxicity issues [3]. For instance, Corexit', the most well known chemical dispersant, used in huge amount (more than two million gallons) in Deepwater Horizon oil spill caused sever environmental concers. This is because the solvent and the surfactants used in its formulation are mostly toxic and nonbiodegradable [3],[4]. Enhancement in environmental awareness and strict governmental legislation has motivated the researchers to identify dispersants/formulations that are safe and environmentally benign [5],[6].

Ionic liquids (ILs) have garnered great attention as a sustainable material due to its fascinating properties e-g;low flammability, low volatility, and high surface activity [7],[8]. Recently, choline based ILs have gain more attraction due to its low toxicity and high biodegradable nature [9],[10]. These long chain environmentally benign surfactants utilized in different fields including; biomass pre-treatment, adsorption of carbon dioxide, demulsifying and emulsifying agents [9],[11],[12]. The ongoing development on the employment of choline based ILs in diverse fields embarks a better understanding about its impact on environment. Therefore, to further explore its safe nature in marine environment, in this study we evaluated the acute toxicity of three choline based ILs, choline laurate [Cho][Lau], choline myristate [Cho][Myr] and choline oleate [Cho][Ol] against the *Danio rerio* fish. Furthermore, to ensure its application for oil spill remediations the aggregation behaviour of ILs were also evaluated. However, it is reported that the micelles produced by a single IL surfactant require a high surfactant content to form a stable micelle [13]. Therefore, to overcome this limitation the aggregation behaviour of the binary mixtures was evaluated to enhance the target properties of the selected system.

2. Experimental

Materials and synthesis of ILs. Lauric acid (Merck, ≥99%), Myristic acid (Sigma, ≥99%), oleic acid (Sigma ≥99%) and choline hydroxide (Sigma, 46 wt% water) were of analytical grade and used to synthesize the respective ILs.

The ILs were synthesized by a developed method [14]. Briefly, respective acids, were added to the choline hydroxide solution (1:1 molar ratio) under stirring. The reaction mixture (under contious stirring) was then refluxed at a temperature of 80 °C for a period of 24 h. Rotary evaporator (80 °C, 2 h) were applied to dry the synthesized ILs and their structure was confirmed by employing^{1}H NMR.

Fish toxicity analyses. A well known method accepted by Organization for Economic Co-operation and Development (OECD) [15] were employed to evaluate the acute toxicity towards zebra fish *(Danio rerio).*The *Danio rerio* were obtained from a fish hatchery (Perak, Malaysia) and screen having size ±2 cm. Further, prior to the toxicity test, safe conditions of survival [16] were fallowed to ensure the optimum health of the fish as shown in Figure 1. The fish were then shifted to 5 L water tanks, connected with air pump that provide sufficient air (1.8 L min^{-1}) for fish survival.

Initially, the test were started with a 100 mg L^{-1} concentration of ILs (called limit test), to know whether the half lethal concentration at 50% mortality (LC_{50}) falls below or above than this value (100 mgL^{-1}). According to the guidelines of OECD [15], the lack of mortality in the limit test depicts that full assay test can be waived. However, to evaluate the actual hazard value, a full test was conducted for ILs surfactants. The obtained LC_{50} values were then compared with a hazard ranking established by Passino and Smith [17].

Surface tension measurements. Surface tension measurements were carried out with high accuracy by employing the pendant drop technique (OCA20, Data Physics, Germany). The pure and mixed system surface tension was evaluated after appropriate mixing of the components. In all experiments, three measurements were carried out and the average value was then reported. The accuracy of the equipment was confirmed by evaluating the methanol and pure water surface tensionat 25 ± 0.1 °C. The uncertainty in achieved results were found to be ± 0.1 mN m^{-1}.

Figure 1. Acclimatization of Zebra Fish *(Danio rerio)* to Confirm Their Optimum Health

3. Results and Discussion

Acute toxicity assessment. Initially, the limit test were performed for all the ILs at a dose of 100 mg L^{-1} for 96 h and it was noticed that LC_{50} values (96 h) of all ILs were found to be higher than100 mg L^{-1}. The obtained results was then compared with the hazard toxicity ranking (Table 1) established by Passino and Smith [17].

Comparing with Table 1, it can be seen that all ILs were fall in ranking of "practically nontoxic" (LC_{50} > 100 mgL^{-1}) towards zebra fish and hence, the complete toxicity test can be waived. However, to strengthen these observations, the full test was carried out for all ILs, e-g; [Cho][Lau],[Cho][Mys] and [Cho][Ol] to determine the exact LC_{50} values after 96 h. The full test was started by selecting five different concentrations as fallows: 600, 700, 800, 850, and 900 mg L^{-1} of [Cho][Lau]. Each dose was poured in separate tank holding 10 fishes. The parameters including: dissolved oxygen, pH and water temperature were measured after every 24 h in order to ensure that the abnormalites are caused by ILs only. The fish activity was checked after every day (24 h) and the number of mortalities were determined after four days (96 h). The LC_{50} can be determined as the concentration at which 50% of the population (fish) died and was found to be 850 mg L^{-1} (Table 2) for pure [Cho][Lau].

In similar way the LC_{50} values of pure [Cho][Mys] and [Cho][Ol] were also evaluated by taking five different concentrations as fallow: 450, 500, 550, 600 and 700 mg L^{-1} and 250, 300, 400, 450 and 550 mg L^{-1} for pure [Cho][Mys] and [Cho][Ol] respectively.

The LC_{50} at 50% mortality was determined to be 600 mg L^{-1} for [Cho][Mys] and 450 mg L^{-1} for [Cho][Ol] (Table 2). This again illustrated that both ILs are classified as "practically nontoxic" according to hazard ranking as shown in Table 1. The above results showed that all three ILs fall in the range of "practically nontoxic" however, each ILs behave differently against the tested organism. Recently, the acute toxicity of [Cho][Lau] merg with biosurfactants against zebra fish was reported. The results illustrates that a practically nontoxic formulation were produced by combining both surfactants [8]. A study performed by Pretty *et al*. [18] observed that ILs caused a different effect towards zebrafish depending on their chemical structure. A similar result was seen in our study where the toxicity of the ILs towards the fish increases by increasing the alkyl chain length of the introducing ILs. Hence, it can be seen that [Cho][Ol] have higher toxicity as compared to the [Cho][Mys] and [Cho][Lau] towards the zebrafish.

Table 1. Toxicity Ranking Established by Passino and Smith [17]

Relative Toxicity	Aquatic LC_{50} (mg/L)
Super Toxic	0.01-0.1
Highly Toxic	0.1-1
Moderately Toxic	1-10
Slightly Toxic	10-100
Practically Nontoxic	100-1000
Relatively Harmless	>1000

Aggregation behavior of mixture comprised of [Cho][Mys] and [Cho][Ol]. The detail aggregation behavior of the binary mixture comprised of [Cho][Lau] and [Cho][Ol] was investigated in our previous study [10] and it was observed that the micelle formed by the binary mixture was more stable as compared to the individual component. Therefore, in this study the agrregation behaviour of the binary mixture comprised of [Cho][Mys] and [Cho][Ol] is investigated in order to check the possible synergism and stability of the mixed system micelles.

Evaluation of critical micelle concentration. The critical micelle concentration (CMC) values of the single components ([Cho][Mys] and [Cho][Ol]) were evaluated and it was observed that the CMC of the single components are similar with those reported in literature [19],[20]. CMC values of the binary mixture with different mole ratios of the components were evaluated and presented in Table 3. The break points of the linear fitting lines of higher and lower concentration regions were used to determine the CMC of individual and mixed system. As depicted in Table 3 that by increasing the [Cho][Ol] mole fraction (α), the values of CMC start decreases and ultimately reached near to the pure [Cho][Ol] CMC value. In the process of micellization, two main factors play their role (i) the hydrophobic part interaction between the component molecules and (ii) the charged heads electrostatic interaction. In the present research study, it is the hydrophobic interaction between the tails of the component molecules that succeeds over the electrostatic interaction and causes micelle formation at lower concentration. Similar trend was observed previouly [21], where the CMCs of the mixed anionic surfactants occurred between the pure components.

Mixed system composition and Interaction parameters. To check the syergism between the components and to investigate that either the mixed surfactant system interaction is ideal or not, several parameters were investigated and discussed in detail. The ideal mixed surfactant system critical micelle concentration can be find out from CMC_1 and CMC_2 of component 1([Cho][Ol]) and component 2 ([Cho][Mys]), respectively by employing the Clint equation [22]as shown in Eq.(1).

$$\frac{1}{CMC*} = \frac{\alpha}{CMC_1} + \frac{(1-\alpha)}{CMC_2} \quad (1)$$

Table 2. Acute Toxicity Estimation of ILs, [Cho][Lau], [Cho][Mys] and [Cho][Ol]

Concentration ($mg\ L^{-1}$) of ILs	Number of fish tested	Mortality (dead fish) after test	% mortality
[Cho][Lau]			
600	10	2	20
700	10	3	30
800	10	4	40
850	10	5	50
900	10	8	80
[Cho][Mys]			
450	10	2	20
500	10	3	30
550	10	4	40
600	10	5	50
700	10	9	90
[Cho][Ol]			
250	10	2	20
300	10	3	30
400	10	4	40
450	10	5	50
550	10	9	90

Table 3. Experimental and Ideal Critical Micelle Concentration of Binary Mixture Comprised of [Cho][Mys] and [Cho][Ol]

α	CMC(mM)	CMC* (mM)	γ_{cmc}(mNm^{-1})
0	6.1	-	29.21
0.2	2.81	3.50	28.14
0.4	1.95	2.46	27.33
0.6	1.57	1.89	26.12
0.8	1.42	1.54	27.86
1.0	1.30	-	30.11

where CMC* is the ideal mixed state CMC. The experimental CMC's values as shown in Table 3 for the mixed system are lower than the ideal *CMC** and illustrating negative deviation (Figure 2) as predicted by the Clint model. This trend shows that the micelle formed at a concentration lower than the ideal mixing state micelle formation, and further illustrates that the interactions between the components are supportive for mixed system micelle formation.

The [Cho][Ol] mole fraction in ideal state (X_{ideal}) of mixed surfactant system was determined using Eq.(2):

$$X_{ideal} = \frac{\alpha\, cmc_2}{\alpha cmc_2 + (1-\alpha) cmc_1} \quad (2)$$

Further, the mixed system non-ideal nature was also confirmed by Holland and Rubingh method [23]. The micellar mole fraction (X_1) of first component ([Cho][Ol]) and interaction parameter (β) between the components was determined by using Eq.(3) and Eq.(4) respectively.

$$\left[\frac{X_1^2 \ln\left(\frac{cmc\alpha}{cmc_1 X_1}\right)}{(1-X_1)^2 \ln\left(\frac{cmc(1-\alpha)}{cmc_2(1-X_1)}\right)}\right] = 1 \quad (3)$$

$$\beta = \frac{\ln\left(\frac{cmc\alpha}{cmc_1 X_1}\right)}{(1-X_1)^2} \quad (4)$$

For the binary mixed system, all the calculated values of X_{ideal}, X_1, and β are presented in Table 4. It can be seen (Table 4 and Figure 3) that both X_{ideal} and X_1 values increases by increasing the [Cho][Ol] mole fractions. The rise of X_1 with [Cho][Ol]concentration (Figure 3) provide an indication that the mixed system micelle formation is favored by enhancing the [Cho][Ol] content.

The nature and extent of interaction between the mixed surfactant system components is evaluated by determing interacting parameter (β) as depicted in Table 4. It is reported that a positive and negative value of β representing an antagonistic interaction, i.e., the molecules interaction are weak and synergistic interaction (strong interaction between molecules), whereas a zero value of β shows no interaction, illustrating ideal behavior [24]. In the present study, all the values of β are negative (Table 4) of the binary mixture, which shows synergistic interactions between the component molecules e-g; [Cho] [Mys] and [Cho][Ol]. The results presented in this study are in line with previous study where the ILs mixed system showed synergism between the components [25].

The activity coefficients (f_1 and f_2) for the mixed surfactant systems were evaluated by using Rubingh [26] equations as stated below.

$$f_1 = \exp\{\beta(1 - X_1)\} \quad (5)$$

$$f_2 = \exp\{\beta X_1^2\} \quad (6)$$

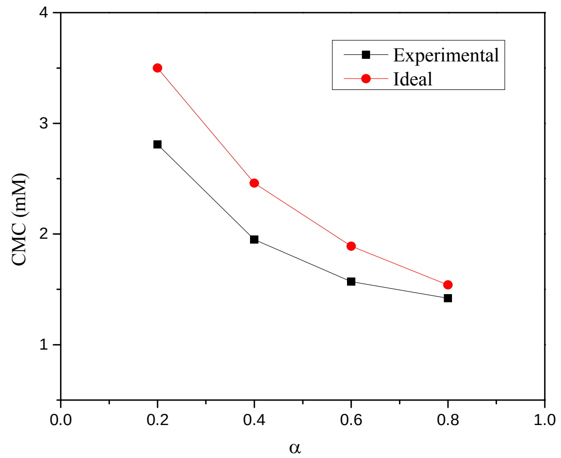

Figure 2. Comparision of Experimental and Ideal CMCvalues of Binary Mixtures

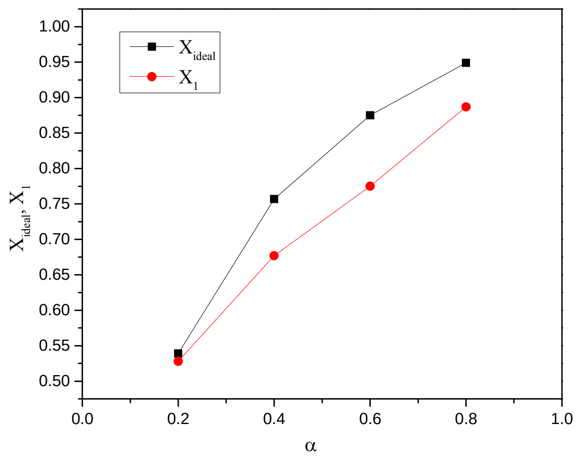

Figure 3. Variation of X_1 and X_{ideal} with Different β Values

The values of activity coefficients f_1 and $f_2$less than unity illustrating the non-ideal system (synergistic behavior). In the existing study, all the values of f_1 and f_2 as shown in Table 4 are less than one for all ratios of mixed system, showing the attractive interaction (synergistic) between the component molecules, i.e., [Cho][Mys] and [Cho][Ol]. All the above results i.e., negative β values and f_1 and f_2 values < unity representing the non ideal nature of the binary system.

Thermodynamics of micellization. Two important thermodynamic parameters including; standard Gibbs free energy of micellization (ΔG^o_{mic}) and excess free energy of micellization (ΔG_{ex}) were evaluated to get an idea about the thermodynamic stability of the micelle formed by the mixed system.

The ΔG^o_{mic} was find out by using Eq.(7) [27].

$$\Delta G^o{}_{mic} = RT ln X_{cmc} \quad (7)$$

where X_{cmc} representing the *cmc* in mole fraction units. All the ΔG^o_{mic} values as shown in Table 5 are negative and this negative trend increases by enhancing the mole ratio of [Cho][Ol]. This further, depicts that the micelle formation is favored and the micellization process becomes more spontaneous by increasing the concentration of [Cho][Ol]. A similar trend was also observed for the binary mixtures of anionic surfactants i-e; sodium dodecyl benzene sulfonate (SDBS) and sodium dodecylsulfate (SDS) [21].

The stability of micelles formed by the mixed system was also confirmed by determining the ΔG_{ex} values using Eq.(8) [28].

$$\Delta G_{ex} = RT[X_1 ln f_1 + (1 - X_1) ln f_2] \quad (8)$$

It is well reported that for ideal mixing, the value of ΔG_{ex} is zero[25], showing no interaction between the components. However, in current work, the values of ΔG_{ex} as presented in Table 5is not zero, showing the possible interactions between the components and their

Table 4. Micellar Composition (X_1 andX_{ideal}), Activity Coefficients (f_1 and f_2) and Interacting Parameter (β) of Binary Mixtures

α	X_{ideal}	X_1	β	f_1	f_2
0.2	0.539	0.528	-0.897	0.818	0.778
0.4	0.757	0.677	-1.157	0.886	0.588
0.6	0.875	0.775	-1.327	0.935	0.450
0.8	0.949	0.887	-1.170	0.985	0.398

Table 5. Thermodynamic Parameters of Binary Mixture with Different β at a Temperature 25 °C

α	ΔG^o_{mic}(kJ.mol^{-1})	ΔG_{ex} (kJ.mol^{-1})	π_{cmc} (mNm^{-1})
0.2	-24.51	-0.556	43.96
0.4	-25.42	-0.628	44.77
0.6	-25.96	-0.574	45.98
0.8	-26.20	-0.291	44.24

stability can be confirmed by the negative value of ΔG_{ex}. Hence, it can be seen (Table 5) that for all mole ratios, the negative ΔG_{ex} values further, proves that the binary system micelles are more thermodynamically stable as compared to the micelle formed by the pure components. Recently, some researchers observed similar results, where thermodynamically stable micelles were noticed for the mixed surfactant system involving ILs [25, 29]. Moreover, π_{cmc}, which shows the reduction of surface tension at CMC, was also determined ($\pi_{cmc} = \gamma_o - \gamma_{cmc}$), where γ_o and γ_{cmc} are surface tension of pure water and surface tension of mixture at CMC. It was observed that thermodynamically stable micelles are able to reduce the surface tension of solvent to lower values (Table 5).

4. Conclusions

In this study the acute toxicity and aggregation behavior of choline-based surface active ILs were investigated. The toxicicty study on zebra fish indicates that all the ILs could be classified as "practically nontoxic" with LC_{50} values greater than 100 mg L^{-1}. However, the full toxicity analysis on zebra fish indicated that the toxicity increases by increasing the anion chain length of the ILs. Therefore, [Cho][Lau] was observed to be least toxic (LC_{50}= 850 mg L^{-1}) where as [Cho][Ol] was the most toxic having LC_{50} values of 450 mg L^{-1}. The aggregation behavior of mixed system composed of [Cho][Mys] and [Cho][Ol] suggested strong synergism between the components and stable micelles produced by the binary surfactant system as compared to the micelles composed of pure components. Furthermore, thermodynamic studies showed spontaneous and thermodynamically stable micelles formation by the mixed surfactant system.

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