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Ionic Liquid and Tween-80 Mixture as an Effective Dispersant for Oil Spills: Toxicity, Biodegradability, and Optimization

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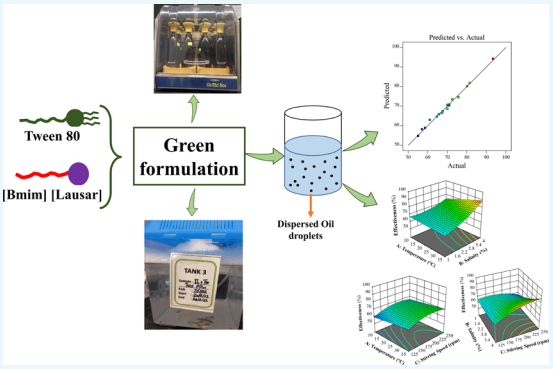
ABSTRACT: Chemical dispersants are used extensively for oil spill remediation. Most of these dispersants are composed of a mixture of surfactants and organic solvents, which raises concerns about aquatic toxicity and environmental impact. In this study, the toxicity and biodegradability of an oil spill dispersant composed of the surface-active ionic liquid 1-butyl-3-methylimidazolium lauroyl sarcosinate [Bmim][Lausar] and Tween-80 were investigated. In addition, important environmental factors including salinity, temperature, and wave-mixing energy were optimized to obtain maximum dispersion effectiveness. The acute toxicity against zebrafish (*Danio rerio*) showed that the developed dispersant was practically non-toxic with a median lethal dose of more than 100 mg L^{-1} after 96 h. The dispersant also demonstrated outstanding biodegradability of 66% after 28 days. A model was developed using a response surface methodology that efficiently (R^2 = 0.992) related the salinity, temperature, and wave-mixing energy of seawater to dispersion effectiveness. The system was then optimized, and a high dispersion effectiveness of 89.70% was obtained with an experimental error of less than 2%. Our findings suggest that the surface-active ionic liquid and Tween-80 mixture could be a viable alternative to toxic chemical dispersants for oil spill remediation.



1. INTRODUCTION

Several oil spill accidents have happened in recent decades because of anthropogenic activities such as drilling for oil, industrialization, and tanker accidents. Among recent oil spills, the Deepwater Horizon and the Sanchi tanker incidents resulted in almost 0.8 and 0.1 billion liters of crude oil, respectively, entering the marine environment.[1] Oil spills are detrimental to aquatic ecosystems and can lead to irreversible environmental losses.[2,3] Several physical, chemical, physicochemical, and biological techniques have been used for remediation of oil spills. Chemical techniques are the most appropriate of these because they are highly efficient and cost-effective.[4,5] Oil spill dispersion with chemical dispersants is a controversial issue because of the toxicity of chemical dispersants.[6] Among the chemical dispersants, Corexit is the most prevalent. During the Deepwater Horizon oil spill, around 7.9 million liters of Corexit was used.[7] Some studies have reported that petroleum distillates and other hydrocarbons found in Corexit have adverse effects on the marine environment.[8] For example, dioctyl sodium sulfosuccinate (DOSS), a surfactant used in Corexit, is toxic and non-biodegradable. Hence, many government agencies have restricted the use of chemical dispersants for oil spill remediation.[9] Therefore, it is necessary to formulate less toxic and environmentally friendly formulations for oil spill remediation.

Several studies have been carried out to identify dispersants that are less toxic and have high emulsifying capabilities compared with conventional commercial dispersants. For instance, researchers have examined the potential of surface-active ionic liquids (SAILs), which are new green surfactants such as oil spill dispersants.[10] Baharuddin et al. successfully formulated an oil spill dispersant composed of five SAILs. This dispersant was both biocompatible and biodegradable and showed more than 90% effectiveness for oil dispersion.[4] SAILs are very effective oil dispersants when they are blended with biosurfactants.[11] In terms of crude oil dispersion, satisfactory outcomes have been achieved, and the dispersants are classed as "practically non-toxic".

To identify environmentally friendly formulations for oil spill remediation, scientists have also used food-based surfactants for crude oil dispersion.[12,13] It has been reported

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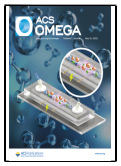

Table 1. Acute Toxicity Assessment of the Binary Mixture of the Dispersant

	test fish number			number of dead fish after the test				
concentration (mg L^{-1})	R1	R2	R3	R1	R2	R3	% mortality	SD
500	10	10	10	0	0	0	0	0
600	10	10	10	2	2	2	20	0
700	10	10	10	5	4	5	46	0.58
750	10	10	10	5	5	5	50	0
800	10	10	10	9	9	8	87	0.58
900	10	10	10	10	10	10	100	0
positive control	10	10	10	10	9	10	97	0.58
negative control	10	10	10	0	0	0	0	0

that a blend of Tween-80 and lecithin is an efficient oil spill dispersant. Recently, we formulated a novel environmentally benign dispersant for oil spill remediation by mixing the food-based surfactant Tween-80 with the SAIL 1-butyl-3-methylimidazolium lauroyl sarcosinate [Bmim][Lausar]. The resulting aggregation behavior and crude oil dispersion were impressive, and the newly developed formulation effectively dispersed the Arab crude oil at a rate of 81.19%.[14] The toxicity and biodegradability of this dispersant need to be evaluated to ensure its safe utilization in the marine environment.[15,16] To prevent irreversible harm and disturbance of the natural ecosystem, the applied dispersant must be biodegradable.[17,18] Alicyclic SAILs containing morpholinium and piperidinium cations have shown 100% biodegradation after 28 days of testing.[19] Knowledge of the effect of crucial environmental variables on the developed formulation and subsequent optimization to achieve maximum dispersion effectiveness will enhance the understanding of the practical implementation of formulation in different marine environments. Earlier studies have highlighted the effect of crucial environmental variables on dispersion effectiveness.[20,21] Many researchers have established correlations that link several environmental factors with dispersion effectiveness. However, in most cases, the difference between experimental and predicted values is high. Hence, it is important to use a comprehensive optimization method that effectively links the dispersion effectiveness with different environmental variables. In this regard, response surface methodology (RSM) is a well-established statistical technique widely used to optimize several processes.[22,23] However, very few studies have used RSM optimization for the dispersion of oil under different environmental conditions. It is highly desirable to optimize the dispersion process to improve its effectiveness and design a model that efficiently determines the influence of different environmental variables on the performance of newly developed formulations.

In this research, we investigated the acute toxicity and biodegradability of an oil spill dispersant containing [Bmim][Lausar] and Tween-80 to evaluate its safety profile. We applied RSM to create a model that linked the dispersion effectiveness to environmental variables, such as the temperature, salinity, and wave-mixing energy of seawater. Finally, the system was optimized using the RSM model for maximum dispersion, and then the developed model was validated experimentally to ensure its precision in determining the effectiveness of the developed formulation.

2. RESULTS AND DISCUSSION

2.1. Acute Toxicity of the Dispersant. Fish toxicity experiments were performed using the [Bmim][Lausar] and Tween-80 dispersants at a concentration of 100 mg L^{-1} (limit test). The median lethal concentration (LC_{50}) was higher than 100 mg L^{-1}, which indicated that the dispersant was non-toxic. Next, experiments were conducted to determine the exact toxicity of the dispersant. Six different dispersant concentrations (500, 600, 700, 750, 800, and 900 mg L^{-1}) were chosen and separately added to a different tank, each containing 10 fish. The data represent the average of the three experiments. Additionally, two control experiments (positive control and negative control) were also observed (OECD Guideline 203). Every 24 h for 4 days, the fish were examined for mortality. The LC_{50} of the dispersant was 750 mg L^{-1} (Table 1), and it was ranked as "practically non-toxic". According to Passino and Smith,[24] materials can be classed as "relatively harmless" if the LC_{50} is less than 1000 mg L^{-1}, "practically non-toxic" if the LC_{50} is between 100 and 1000 mg L^{-1}, "slightly toxic" if the LC_{50} is between 10 and 100 mg L^{-1}, "moderately toxic" if the LC_{50} is between 1 and 10 mg L^{-1}, and "highly toxic" if the LC_{50} is between 0.1 and 1 mg L^{-1}.

Many researchers have evaluated the toxicity of Tween-80 toward different organisms. For instance, Ali et al. determined that the LC_{50} for Tween-80 in zebrafish (*Danio rerio*) embryos was 323 mg L^{-1} after 96 h.[25] Zenati et al. observed that Tween-80 was non-toxic toward Artemia nauplii.[26] Similarly, Tween-80 was not toxic toward 18 distinct fungal species.[27] Recently, our group evaluated the toxicity of [Bmim][Lausar] with other surfactants toward zebrafish (*D. rerio*) and grouper fish (Epinephelinae) and found that it was practically non-toxic with a LC_{50} of 173.78 mg L^{-1}.[4] The toxicity of the developed dispersant was also compared with commercial dispersants. The toxicity of Finasol OSR 52 was evaluated against different marine species. Finasol had a mean LC_{50} of 43.27 mg L^{-1}.[28] Similarly, Pie and Mitchelmore[29] recently studied the toxicities of different chemical dispersants and found that all the dispersants were slightly toxic after 48 h of exposure. The LC_{50} of Corexit 9500 (55 mg L^{-1}), Petro-Clean (52 mg L^{-1}), Orca (76.5 mg L^{-1}), and Dispersit SPC 1000 (10.1 mg L^{-1}) was indicative of their toxic natures. Additionally, the obtained results were compared with those of the other dispersants published recently for oil spill remediation. For instance, Shah et al. 2021[30] determined the toxicity of the binary mixture of lactonic sophorolipid (LS) with choline myristate [Cho][Mys] and choline oleate [Cho][Ol]. The results showed that both dispersants were found to be non-toxic with LC_{50} values of 670 and 500 mg L^{-1}, respectively. Hence, the dispersant we developed has low toxicity compared with commercial and latest dispersants. However, a precise toxicity comparison is not possible because of differences in the procedures and organisms used for the toxicity evaluations. Importantly, our dispersant does not contain any organic solvent or hazardous

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Table 2. Biodegradability Analysis of the Dispersant Binary Mixture and Pure Components

test materials	R1	R2	R3	% biodegradation ± SD[a]	% biodegradation[b]
binary mixture	63.72	70.16	67.17	67.02± 1.07	66.00
[Bmim] [Lausar]	61.02	60.76	60.59	60.85 ± 1.02	60.82
Tween-80	70.61	71.08	71.20	70.99 ± 1.03	70.98
reference[c]	93.12	94.97	95.78	94.63 ± 1.36	93.98

[a]The average biodegradation of three replicates. [b]Calculated using the formulas in eqs 1−3. [c]Sodium acetate was used as a reference material.

Table 3. Experimental and Predicted Values of the Design Obtained by RSM-FCCCD

					response	
		factor 1	factor 2	factor 3	% effectiveness	
run	space type	*A*: temperature (°C)	*B*: salinity (%)	*C*: stirring speed (rpm)	predicted	experimental
1	factorial	10	4	125	58.70	58.90
2	factorial	35	4	250	93.45	94.16
3	axial	22.5	2.5	250	70.05	68.64
4	axial	22.5	1	187	65.73	65.84
5	axial	35	2.5	187	72.60	73.44
6	center	22.5	2.5	187	70.50	70.50
7	axial	10	2.5	187	67.70	67.31
8	factorial	35	4	125	75.72	74.54
9	center	22.5	2.5	187	70.15	70.50
10	center	22.5	2.5	187	70.72	70.50
11	factorial	10	4	250	80.20	80.14
12	center	22.5	2.5	187	70.90	70.50
13	axial	22.5	4	187	81.44	81.78
14	factorial	35	1	125	64.71	64.66
15	center	22.5	2.5	187	70.67	70.50
16	axial	22.5	2.5	125	61.09	62.95
17	factorial	10	1	250	57.08	58.15
18	center	22.5	2.5	187	70.98	70.50
19	factorial	10	1	125	67.23	66.41
20	factorial	35	1	250	55.09	54.78

Table 4. ANOVA Results of the Quadratic Model

source	sum of squares	*df*	mean square	*F*-value	*p*-value	
model	1470.49	9	163.39	149.82	<0.0001	significant
A-temperature	94.00	1	94.00	86.20	<0.0001	
B-salinity	634.73	1	634.73	582.02	<0.0001	
C-stirring Speed	80.77	1	80.77	74.06	<0.0001	
AB	151.21	1	151.21	138.65	<0.0001	
AC	1.31	1	1.31	1.20	0.2984	
BC	435.12	1	435.12	398.99	<0.0001	
A^2	0.05	1	0.05	0.043	0.8395	
B^2	30.02	1	30.02	27.53	0.0004	
C^2	61.03	1	61.03	55.96	<0.0001	
residual	10.91	10	1.09			
lack of fit	10.46	5	2.09	23.28	0.1898	not significant
pure error	0.44	5	0.09			
core total	1481.40	19				

material and can be considered safe for use in the aquatic environment.

2.2. Biodegradability of the Dispersant. Investigation of biodegradability is important as it can be used to determine the elimination of persistent compounds from the environment.[31] Biodegradation is described as the degradation of organic matter by microorganisms (e.g., fungi, protozoa, and bacteria) producing CO_2 and water under aerobic conditions. Biodegradation results in a significant reduction in the molecular weight and the number of carbon atoms in the molecular formula.[32]

In this study, the biodegradability study of the pure components (Tween-80 and [Bmim][Lausar]) and the dispersant (60:40 (w/w) mixture of [Bmim][Lausar] and Tween-80) was carried out using closed bottle tests. The OECD recommends that a substance is classified as "biodegradable" if 60% of the initial amount degrades in 28 days under aerobic conditions.[33] The results from the current study (Table 2) showed that the degradation rates of pure

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[Bmim][Lausar] and Tween-80 were 60.82 and 70.98%, respectively. These findings are consistent with earlier research.[19,34] The degradation rate of the developed dispersant was 66% (Table 2), which illustrates that it is readily biodegradable. The results presented in the current study were compared with those in previous reports. Brakstad et al. examined the biodegradability of DOSS in seawater. Their results showed that the degradation rate of DOSS was 16% after 54 days.[35] Recently, Cai et al. investigated the biodegradability of Corexit 9500A and observed that it had low biodegradability (10−20%) after 30 days.[36] Similarly, Prince et al. investigated the biodegradation of three commonly used commercial dispersants: Corexit 9500A, Dasic Slickgone, and Finasol OSR52. Their results revealed that the degradation rate for each of the three dispersants was 32% after 28 days.[37] Therefore, we can infer that our dispersant is more biodegradable than conventional dispersants.

2.3. Statistical Analysis and Model Development. The software Design Expert (version 11) was used to develop a design matrix (Table 3). More than three replicates were performed to investigate the experimental errors. Afterward, a response prediction model was established, and the data (Table S3) were used to select an appropriate model. Quadratic, cubic polynomial, two-factor interaction, and linear models were suggested.

Among these, the quadratic model was the best suited to predict the response. Compared with the other models, the quadratic model was statistically significant because it addressed the maximum variation in the response (dispersion effectiveness). Analysis of variance was used to determine the quadratic model's significance and fitness. The model was significant and had a low p-value ($p < 0.0001$) and a high F-value (149.82) (Table 4). Additionally, a non-significant lack of fit showed that the quadratic model fit the data.

The results from the model showed that individual factors of temperature (A), salinity (B), and stirring speed (C), and combined factors AB, BC, and C^2 were significant, whereas AC, A^2, and B^2 were insignificant. The high F-value (582.02) of the salinity showed that the salinity had a larger effect on oil dispersion than the temperature or stirring speed. The standard deviation was 1.04, and the coefficient of variance was 1.50%. The R^2 was 0.9926, which indicated that the model was a good fit for the data points.[38] However, adding more data points can change the R^2 value because of model overfitting, and adjusted (0.9937) and predicted (0.9902) R^2 values can be used to prevent overfitting. In the current study, the difference between the adjusted and predicted R^2 values was less than 0.2, which demonstrated that the selected quadratic model had high accuracy.

The experimental values obtained from the FCCCD were used to establish the quadratic equation for the response (Y) (eq 1).

$$Y = 57.26743 - 0.199485A - 21.99809B + 0.312717C + 0.231867AB - 0.000518AC + 0.078667BC - 0.000838A^2 + 1.46848B^2 - 0.001206C^2 \quad (1)$$

The limits of the above quadratic model (eq 1) for the temperature, salinity, and stirring speed were 10 °C < A < 35 °C, 1% < B < 4%, and 125 rpm < C < 250 rpm, respectively.

Several diagnostic tests were performed to confirm the accuracy and applicability of the proposed quadratic model (eq 1). A normal probability graph of the residuals (Figure S1A) illustrated their normal distribution and confirmed the model validity. The actual and predicted plots (Figure S1B) showed good agreement. Therefore, the quadratic model performs well in predicting the response. Additional confirmation of the model validity was obtained by plotting the residuals against the expected dispersion effectiveness (Figure S1C). The experimental values were distributed inside the residual area, which indicated that the error was negligible. Hence, the proposed model is suitable to evaluate dispersion under selected environmental conditions.

2.4. Effects of Variables and Their Interactions. Three-dimensional diagrams are very effective for illustrating the effect of independent variables on the response. Figure 1 illustrates the effects of salinity, temperature, and the interaction of these variables on the dispersion effectiveness. The temperature significantly affected the dispersion efficiency, and as the temperature of simulated seawater was increased from 10 to 35 °C, the dispersion effectiveness increased. This is because the viscosity of oil is low at elevated temperatures, which results in better dispersion. Similar results have been reported previously. For example, Li et al.[39] observed that dispersion effectiveness increased at a higher temperature. Furthermore, Shah et al.[20] also noticed a considerable increase in dispersion effectiveness with an increase in the temperature of seawater.

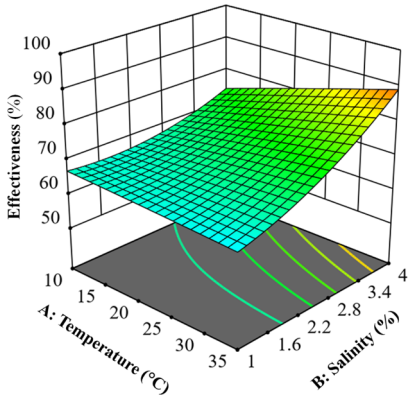

Figure 1. Effect of salinity and temperature on dispersion effectiveness with a constant stirring speed of 187 rpm.

The dispersion effectiveness also increased with increasing salinity (Figure 1), and the effect of salinity was more prominent than that of the temperature. This is because high salinity reduces the solubility of surfactant molecules in water.[40] Hence, the contact between surfactant molecules and crude oil increased at high salinity, and this improved the dispersion effectiveness. Our results are consistent with those reported previously. Shah et al. determined the effect of salinity on dispersion efficiency and found that with the increase in salinity, dispersion effectiveness increased.[20] Similarly, Chandrasekar et al.[41] investigated that as the salinity increased, dispersion effectiveness also increased. Interestingly, our formulation performed better in highly saline water than in brackish water (Figure 1); therefore, it was effective in seawater with high salinity. Hence, the net effect was found to be insignificant. The combined effect of temperature and salinity was insignificant ($p > 0.05$). This is because a higher temperature accelerates weathering of crude oil, which can potentially reduce the effectiveness of the dispersant. Addi-

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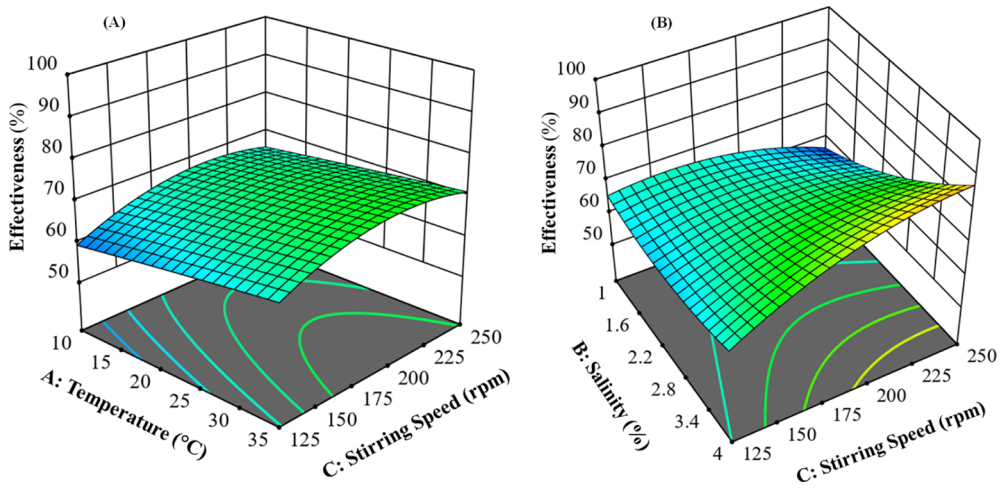

Figure 2. (A) Effect of temperature and stirring speed on dispersion effectiveness at 2.5% salinity and (B) effect of salinity and stirring speed on dispersion effectiveness at 22.5 °C.

tionally, a high temperature also causes the degradation of crude oil, and the high stirring speed further accelerates this degradation.[20]

The three-dimensional graph (Figure 2A) demonstrates the effect of temperature and stirring speed on dispersion effectiveness. The mixing speed had a considerable impact on dispersion effectiveness. This phenomenon was attributed to energy dissipation, which causes tiny eddies that ultimately lead to a better breakdown of crude oil. Many researchers have demonstrated that the crude oil droplet size is reduced with increases in the mixing energy.[4,20]

For example, Li et al.[39] studied the performance of Corexit 9500 with various wave speeds and observed that small oil droplets were produced at high mixing energies. Mukherjee and Wrenn[42] conducted laboratory experiments and confirmed the production of small oil drops at a high speed. Some recent studies[20,43] have shown that high mixing speed improve the dispersion effectiveness. However, the combined effect of temperature and the stirring speed was insignificant (p = 0.2984).

Figure 2B illustrates the influence of stirring speed and salinity on the response (dispersion effectiveness). The effectiveness clearly increased with increases in both the salinity and the stirring speed. Interestingly, the combined effect of the stirring speed and salinity was significant (p < 0.0001). This could be attributed to the dispersant of small droplets at a high stirring rate and the reduction in the solubility of the dispersant at high salinity in simulated seawater, which ultimately results in high dispersion. Similarly, Saleh et al. 2021[44] investigated the dispersion effectiveness of a novel dispersant synthesized from fish and lobster waste and observed that the dispersion effectiveness was improved by increasing the stirring speed from 200 to 250 rpm. Similarly, Shah et al. 2020[20] reported that the dispersion effectiveness was increased with the increase in salinity.

2.5. Optimization of the Process by RSM. An optimization study was carried out to find the maximum dispersion. To obtain the maximum dispersion, three variables (temperature, salinity, and stirring speed) were set in the range of lower and upper boundaries (Table S4A). The optimum parameters for the maximum dispersion according to the RSM-FCCCD were a temperature of 33.95 °C, a salinity of 3.69%, and a stirring speed of 218 rpm. Under the optimized conditions, 89.70% dispersion effectiveness was achieved with a desirability value of 0.972 (Table S4B). The result presented in the current study showed that at optimized conditions using RSM-FCCCD, high effectiveness is achieved as compared to the early studies performed without optimization. For instance, the study performed by Zhu et al.[45] achieved 77% of the dispersion effectiveness for the ANS crude blend by mixing the fish-based lipopeptide bio dispersant with DOSS. Similarly, Nawavimarn et al.[46] found that by using lipopeptides as a biosurfactant with a non-ionic oleochemical, Dehydol LS7TH, the dispersion efficacy of the Arabian light crude oil was achieved by 74.8%. Hence, these findings indicated that our formulation under optimized conditions has higher effectiveness under different environmental conditions.

2.6. Model Validation. Additional experiments were conducted under similar conditions to those in Table S4B to ensure the validity of the optimized conditions developed by FCCCD. The experimental results were found to be identical to the predicated results, confirming the accuracy of the quadratic model proposed by the software. The percentage error between the experimental and predicted results for dispersion effectiveness was found <2.0% in all sets of experiments (Table 5). The small error demonstrated that the developed model is reliable for the evaluation of the dispersion effectiveness of the dispersant under different environmental conditions.

Table 5. Optimum Parameters and Validation of the Experimental Results

	effectiveness		
run	experimental	predicted	% error
1	88.05	89.70	1.93
2	88.64	89.70	1.20
3	87.54	89.70	2.46
4	88.72	89.70	1.11
5	88.92	89.70	0.88
mean			1.64
standard deviation			0.56

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3. CONCLUSIONS

We investigated the acute toxicity and biodegradability of a dispersant composed of Tween-80 and [Bmim][Lausar]. The acute toxicity analysis categorizes the dispersant formulation as "practically non-toxic" with an LC_{50} value of 700 mg L^{-1}. The biodegradability results revealed that the formulation is readily biodegradable, with a biodegradability value of 66%. Moreover, the system was optimized using RSM, and a model was developed that effectively correlated the environmental factors with dispersion effectiveness. To obtain the maximum dispersion, three variables (temperature, salinity, and stirring speed) were selected, and under optimized conditions, maximum dispersion effectiveness of 89.70% was achieved. Overall, the developed dispersant has lower toxicity, good biodegradability, and higher oil spill dispersion effectiveness. The results revealed that dispersant formulation is environmentally friendly and could efficiently remove oil spills under various environmental conditions.

4. EXPERIMENTAL SECTION

4.1. Materials. Tween-80 (polyoxyethylene sorbitan monooleate, ≥99%) was obtained from Sigma-Aldrich (Waltham, MA). 1-Butyl-3-methylimidazolium lauroyl sarcosinate [Bmim][Lausar] was prepared using a metathesis reaction established by Mustahil et al.[19] Dichloromethane (DCM), NaCl, $MgCl_2$, and Na_2SO_4 (purity > 98%) were obtained from Merck (Germany). Zebrafish (*D. rerio*) were purchased from an aquatic store in Seri Iskandar, Malaysia. Arab crude oil with a density of 902 g L^{-1} and a viscosity of 5.34×10^{-5} m^2 s^{-1} at room temperature was acquired from the Petroleum Industry of Malaysia Mutual Aid Group Sdn Bhd (PETRONAS). Stimulated seawater samples were prepared by an established method.[12] Details of the procedure are given in Table S5.

4.2. Preparation of the Oil Spill Dispersant. A 60:40 (w/w) mixture of Tween-80 and [Bmim][Lausar] was prepared in water. This ratio was selected because it gave the highest effectiveness in our previous study.[14] The total surfactant mass fraction was 5%.

4.3. Acute Fish Toxicity Analysis. The acute toxicity of the dispersant was evaluated against zebrafish (*D. rerio*) using an approved procedure established by the Organization of Economic Co-operation and Development (OECD Guideline no. 203).[47] Zebrafish (*D. rerio*) are a well-known laboratory model species that are widely used for toxicity studies.[48] For this study, zebrafish (*D. rerio*) were selected that were similar in length (±2 cm). Specific parameters (dissolved oxygen level of 90%, pH 8.2, and temperature of 23 ± 1 °C) for their survival were maintained before the toxicity evaluation.[49,50] After limit testing with a dispersant concentration of 100 mg L^{-1}, the toxicity of the dispersant was determined using concentrations higher than 100 mg L^{-1}. A total of six samples at different dispersant concentrations were taken, and 10 fish for each concentration (triplicate setup) were taken for toxicity analysis. Two controls were taken: one was dechlorinated tap water (negative control) and the other was (positive control) with 3,4-dichloroaniline (3,4-DCA) with the concentration of 4 mg L^{-1} as recommended in the OECD Guideline 203.

4.4. Biodegradability Analysis. The biodegradability of the developed dispersant was evaluated using closed-bottle tests.[4] First, a seeding water sample was collected from the gas district cooling plant at the Universiti Teknologi PETRONAS, Malaysia. Brown glass bottles (500 mL) filled with a mineral salt medium, inoculum (bacterial concentration: 10^6 cell/mL), and test substances (concentration: 10 mg L^{-1}) were placed in an OxiTop system (WTW GmbH, Weilheim, Germany). The experiments were performed in duplicate for 28 days at 20 °C. Blank experiments were conducted without test substances. The amount of dissolved oxygen in each bottle determined the degradation rate. The blank sample oxygen demand was used to adjust the degradation rate for the theoretical oxygen demand (TheOD), which was determined using the following equation

$$\mathrm{TheOD} = \left\{16\left[2\mathrm{C} + \frac{1}{2}(\mathrm{H} - \mathrm{Cl} - 3\mathrm{N}) + 3\mathrm{S} + \frac{5}{2}\mathrm{P} + \frac{1}{2}\mathrm{Na} - \mathrm{O}\right] \mathrm{mg/mg}\right\}/\mathrm{MW} \quad (2)$$

The biological oxygen demand (BOD) and degradation rate were determined using eqs 3 and 4, respectively.

$$\mathrm{BOD} = \{\mathrm{O_2\ uptake\ by\ the\ test\ substance\ in\ mg/L} - \mathrm{O_2\ uptake\ by\ blank\ in\ mg/L}\}/\{\mathrm{test\ substance\ in\ bottle\ mg/L}\} \quad (3)$$

$$\%\ \mathrm{degradation} = \frac{\mathrm{BOD}(\mathrm{mgO_2/mg\ test\ substance})}{\mathrm{the\ OD}\ (\mathrm{mgO_2/mg\ test\ substance})} \times 100 \quad (4)$$

4.5. Baffled Flask Test. A baffled flask test was used to determine the dispersion effectiveness.[51] In all experiments, after adding 120 mL of the simulated seawater to a baffled flask, 100 μL of Arab crude oil was poured directly on the water surface. Next, 4 μL of the dispersant was transferred immediately onto the surface of the oil, which gave a dispersant to oil ratio of 1:25. The samples were mixed in the orbital shaker at 200 rpm for 10 min and stabilized. The oil was extracted from the water solution (30 mL) using DCM, and the absorbance was measured at 340, 370, and 400 nm by spectrophotometry.[51] Absorbance and wavelength graphs were plotted, and the area (A) was calculated using eq 5.

$$A = \frac{(\mathrm{Abs_{340}} + \mathrm{Abs_{370}}) \times 30}{2} + \frac{(\mathrm{Abs_{370}} + \mathrm{Abs_{400}}) \times 30}{2} \quad (5)$$

where $\mathrm{Abs_{340}}$, $\mathrm{Abs_{370}}$, and $\mathrm{Abs_{400}}$ are the absorbance values at 340, 370, and 400 nm, respectively. The result from eq 5 was then used to calculate the quantity of oil dispersed in the simulated seawater in eq 6.

$$T_{\mathrm{od}} = \frac{A}{S_{\mathrm{cc}}} \times V_{\mathrm{DCM}} \times \frac{V_{\mathrm{tw}}}{V_{\mathrm{ew}}} \quad (6)$$

where T_{od} is the amount of dispersed oil (g L^{-1}), S_{cc} is the slope of the calibration graph, V_{DCM} is the extracted DCM volume (mL), V_{tw} is the simulated seawater volume (mL), and V_{ew} is the volume of extracted seawater. The effectiveness was determined by dividing the volume of oil dispersed in the simulated seawater by the overall oil volume added to the baffled flask.

$$\mathrm{effectiveness} = \frac{T_{\mathrm{od}}}{\rho_{\mathrm{oil}} V_{\mathrm{oil}}} \times 100 \quad (7)$$

where ρ_{oil} is the oil density and V_{oil} is the volume of oil applied, respectively.

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4.6. Experimental Design and Optimization. The effects of temperature, salinity, and stirring speed (denoted as A, B, and C, respectively) as independent variables on the dispersion effectiveness were evaluated using Design Expert Software (version 11). RSM in combination with the face-centered central composite design (FCCCD) was used to optimize the entire system. The FCCCD model was used to evaluate the impact of the three variables at high (+1), low (−1), and central (0) levels. To simulate real seawater conditions, the salinity range was varied from 1 to 4% for brackish and highly saline seawater.[52] The temperature of the simulated seawater was varied from 10 to 35 °C to replicate the sea surface temperatures in different regions.[53,54] Similarly, the stirring speed was varied from 125 to 250 rpm to represent the surface layer and breaking wave-mixing energies.[42] The ranges for these variables are shown in Table S6.

The experimental boundary conditions were predetermined; therefore, additional factorial space was not evaluated further. The FCCCD is an ideal design (statistical algorithm) to optimize the system for such a situation. The design matrix (Table 3) was developed using FCCCD. The independent variables were set precisely; however, the stirring speed varied by ±1.0 rpm. The response (dispersion effectiveness) was calculated using the standard baffled flask test.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c00752.

Dispersion effectiveness diagnostic plot; acute toxicity assessment of Tween-80 and SAIL, selected conditions for RSM-FCCCD; and optimization results produced by RSM-FCCCD, preparation of simulated sea water at different salinity (PDF)

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Notes

The authors declare no competing financial interest.

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