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Chicken Feather Protein Dispersant for Effective Crude Oil Dispersion in the Marine Environment

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ABSTRACT: Various studies report that aside from the adverse impact of the crude oil on the marine environment, there is the likelihood that chemical dispersants used on the surface of water as oil-treating agents themselves possess a degree of toxicity, which have additional effects on the environment. To eliminate the subject of toxicity, there exist several materials in nature that have the ability to form good emulsions, and such products include protein molecules. In this study, chicken feathers which are known to contain ≥90% protein were used to formulate a novel dispersant to disperse crude oil in seawater (35 ppt). Protein from chicken feathers was extracted and synthesized into the chicken feather protein (CFP) dispersant using deionized water as a solvent. Emulsions formed from CFP-synthesized dispersants were stable over a considerably long period of time, whereas the droplet sizes of the emulsion formed were on the average very small in diameter, making droplet coalescence very slow. The CFP dispersants exhibited moderate surface and interfacial activity at normal seawater salinity. Using the US EPA's baffled flask test, at 800 and 1000 mg/ml CFP surfactant-to-oil ratios, dispersion effectiveness values of 56.92 and 68.64 vol % were obtained, respectively, which show that CFP has a great potential in crude oil dispersion. Moreover, the acute toxicity test performed on Nile tilapia showed that CFP was practically nontoxic with an LC50 value of more than 100 mg/L after 96 h of exposure. The results obtained showed that the CFP dispersant is environmentally friendly.

1. INTRODUCTION

Taking the last decade into account, over a billion gallons of oil were spilled globally and about six million tonnes of oil were deposited into the ocean annually.[1] There are several methods that are used to reduce the impact of oil spills on the environment, and these include burning in situ, mechanical containment and recovery, application of chemical dispersants, and usage of sorbent materials. Dispersant application is the most suitable emergency response method for large-scale oil spills and spills that are very far from the shore.[2]

When right amounts of chemical dispersants are applied under appropriate conditions, dispersant application can be said to be one of the best oil spill counterresponse measures. Chemical dispersants are homogeneously formed mixtures of surfactants and solvents, which are sprayed onto spilled oil on the water surface with the aim of causing the oil to break up into the water column.[3] With this mechanism, the surface-active ingredients which are amphiphilic in nature are adsorbed at the oil−water interface, and this causes an alteration in the chemical and physical nature of the oil so that the interfacial tension is significantly reduced.[4,5] When this occurs in the presence of adequate mixing energy from cresting or breaking sea waves, the oil breaks up into tiny droplets.[6] The tiny oil droplets diffuse into the water column both horizontally and vertically by wave action and stay within the water column due to the low buoyancy of the tiny droplets, which does not allow them to rise quickly, considering that they constantly get upward movement resistance from the downward motion of the water.[3,7] The tiny droplets of oil have a large surface area, which enhances microbial (bacteria) actions, leading to the consumption of the oil as their source of energy.[8] The oil is consumed by the oil-degrading microbes and converted into less harmful and nonhazardous substances and hence integrated into the natural biogeochemical cycles.[9] When the oil is finally removed from the water surface, it reduces the adverse impact of the spilled oil on the environment.[10]

Despite being potent in removing spilled oil from the water surface into the water column, chemical dispersant application has generated continuous discussions on toxicity effects and its short- and long-term impacts on the environment as a result of their hydrocarbon-based formulations.[11,12]

The famous chemical dispersants used in combating the 2010 Deepwater Horizon incident were Corexit 9527A (which was phased out after weeks of usage) and Corexit 9500A and are reported to have had a moderate health risk/toxicity to spill

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responders and aquatic life.[7,12] Corexit 9500A has been reported to have more toxic effects on plankton, Daphnia, and other species in their early stages of life than to fish, crustaceans, and corals as per aquatic tests.[13]

As per reports, the active ingredients and solvents used to formulate these dispersants are the potential cause of the toxicity effects these dispersants could be carrying.[10,14] Examples of the chemical surfactants and solvents used in the formulation of the dispersants include dioctyl sodium sulfosuccinate salt, 2-butoxyethanol, and propylene glycol, which are chemicals synthesized from hydrocarbon sources.[12,14]

In recent times, researchers have focused on using natural surfactants and other smart but environmentally benign formulations such as using ionic liquids and Pickering emulsions[15,16] with the aim of ending discussions on chemical dispersant toxicity and its effects on the environment. It is perceived that natural products are biocompatible and readily biodegradable and forms stable, nontoxic emulsions; hence, hydrocarbon-based surfactants should be replaced with natural surfactants, which have less or no toxic impact on biodiversity.

These naturally occurring surfactants commonly referred to as emulsifiers are already being used in various industries such as the food, pharmaceutical, and medicinal industries, and proteins can be considered as one. Proteins are used to form emulsions that are used in food formulations and drug and nutrient delivery.[17] Protein molecules are capable of forming good emulsions because they possess both hydrophobic regions and charged hydrophilic regions that get oriented at the oil−water interface and as a result lower the interfacial tension to form stable emulsions.[18−20] Proteins form viscoelastic, adsorbed layers on oil droplets and do not cause them to form coalescence, therefore promoting emulsion stability as well as microbial degradation of the oil.[21,22] Proteins are known to be excellent emulsifiers, and these properties of proteins primarily stem from (a) their ability to significantly reduce the interfacial tension by unfolding and adsorbing at the oil−water interface[4,22] and (b) their ability to form electrostatic, mechanical, and structural barriers, which form resistance against destabilization processes.[17,23] Changes in environmental factors including temperature, ionic strength, and pH affect the surface hydrophobicity of proteins and its affinity toward the interface.[17,24,25] It is therefore necessary to consider the functional properties of proteins such as solubility, gelling, and foaming capacity, which can confirm whether a protein emulsion is stable or not stable.[21,26] Proteins are less expensive, easily accessible, and available in multiple plant and animal sources such as soybean, peas, milk, whey germ protein, fish, meat, and many others.

Notable research studies have been conducted using protein sources in the area of natural organic emulsion formulations. In a study conducted by Saleh et al., sea bream and sea brass mixture, fish waste mixture, and lobster waste were used to formulate dispersants to disperse crude oil spill in saline water and the reported emulsifying activities were 90, 93.3, and 94.5%, respectively, indicating that dispersants formed from these protein sources formed stable oil−in−water (o/w) emulsions.[4]

Zhong et al. also conducted a study using whey protein−puerarin composites to form and determine the interfacial behavior and stability of oil−in−water emulsions. The study concluded that the whey protein−0.5% puerarin mixture formed a high thermally stable emulsion, enhanced storage, and improved surface activity (has an enhanced ability to reduce the interfacial tension of oil−in−water emulsions).[27] Sridharan et al. investigated pea flour, which contains 20 wt % protein and 50 wt % starch as a stabilizer of oil−in−water emulsions. In the study, the interfacial results of the pea flour were similar to those of concentrated pea protein systems (approximately 55 wt % protein), which proves that proteins are the main stabilizers of the oil−water interface. Pea flour showed a typical behavior of amphiphilic proteins at the interface. Interfacial tension was reduced, whereas the elastic and viscous nature of the interface formed by the pea flour indicated a stable emulsion formation.[28]

Feathers from chicken are a very rich source of crude protein and are renewable, biodegradable, biocompatible, and low-cost byproducts of chicken production, which are often thrown away as waste.[29,30] Over 8.5 billion tonnes of chicken feathers are produced from over 24 billion chickens slaughtered every year across the world.[31,32] Chicken feathers have a distinctive hierarchical structure, which makes them viable for various applications including animal feed, fertilizers, additives in lubricants, and natural composites.[33,34] They can also be used in the area of oil spill clean-up as sorbent materials due to its high sorption capacity and low density (about 0.9 g/cm^3).[30,35]

Chicken feather mass contains about 91% protein, 1% lipids, and 8% water components.[34,36,37] The major protein contained in chicken feather is keratin and falls under hard keratin classification.[36] Due to the presence of hard keratin in chicken feathers, they can undergo mechanical stress and various types of thermal and chemical treatment without permanent damage.[38] The native protein structure is stabilized by many factors, which include salt linkages, hydrogen bonds, van der Waals forces, and hydrophobic interaction or the hydration effects of non-polar groups.[39] The pure keratin protein chain is an insoluble, extremely mechanical stable structure that is tightly packed in the α-helix and β-sheets into a supercoiled polypeptide chain.[32,40] Furthermore, feather keratin is made of above 90% β-sheet conformation (β-keratin) with 96 amino acids, which possess seven cysteine residues as terminals[41] as per a study conducted by Arai et al.;[42] nonetheless, the central portion of keratin also contains α-helices.[30,32,43] High levels of cysteine which are found in feather keratin provide disulfide bonds and extensive crosslinkages, which cause high mechanical stability and toughness of proteins in feather.[40] The presence of cysteine in keratin also provides resistance to proteolytic degradation by papain, trypsin, pepsin, and other specific protease.[32,40] However, keratin found in chicken feather can be metamorphosed to natural protein in soluble alkali or acid and digested by pepsin and trypsin, and this can be achieved by breaking down the disulfide bonds of keratin.[44,45] When the disulfide bonds are broken, there is a possible reduction in keratin strength, which makes it soluble and transform into natural protein.[46,47] Although proteins are bulkier with large molecular weights, which makes them diffuse at a slower rate, feather keratin protein has a relatively small molecular weight of 10 kDa and is uniform in size.[36,43] Proteins are slow to be adsorbed at the interface due to their high molecular weights; however, at high concentrations and/or under stirred conditions, protein adsorption is rapid.[17,23]

The aim of this study is to formulate an environmentally benign dispersant from chicken feather protein (CFP), capable of crude oil dispersion in the marine environment. The logic behind selecting chicken feather as a source of emulsifier is because of its rich protein reserve as well as its abundance in

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the environment. Sodium sulfide was used as a reducing agent to decrease the stability of solid form keratin fibers in order to break down the hydrogen bonds, disulfide bonds, and salt linkages in the keratin fibers and dissolve them into the protein solution.[48] It is to be noted that the resulting extract is a true protein solution and did not behave as a product of hydrolysis and neither did the reducing agent cause any chemical changes to the protein obtained.

CFP dispersants were formulated in deionized (DI) water because the use of water as a delivery medium eliminates the usual hydrocarbon solvents used in chemical dispersant formulations, and this will help achieve a nontoxic dispersant formulation. The use of chicken feather protein in this study does not only serve as an effective oil spill dispersant but as a means of feather waste management. The haphazard disposal of feather waste is environmentally unacceptable and can lead to several human ailments including fowl cholera, chlorosis, and avian mycoplasmosis.[32] Burning of feather waste as well results in the production of greenhouse gas emissions, which contribute to global warming and other greenhouse effects.[46] Hence, redirecting feather waste into production of CFP dispersants contributes to effective waste management.

In this study, the dispersion effectiveness (DE) of CFP-formulated dispersants on crude oil was investigated, effects of different salinities on CFP dispersants were also examined, and stability tests, toxicity, surface, interfacial and emulsifying activities, and droplet size distribution were investigated as well.

2. RESULTS AND DISCUSSION

2.1. Protein Extraction Yield. 27 g of solid protein was obtained from the 50 g of chicken feather dissolved in 2 L of 0.5 M sodium sulfide solution representing 54% mass yield. The protein yield from sodium sulfide reduction is relatively high. After the purification process, it was observed that protein is soluble in sodium hydroxide solution.

2.2. Protein Characterization Analysis. To confirm the presence of protein, the product obtained was characterized by the Biuret test, absorbance test, Fourier transform infrared spectroscopy analysis (FT-IR), and NMR spectroscopy. The Biuret test was performed for the protein product, and the change in color was observed to be purple (as indicated in Table 1) after the reagent was added, indicating the presence of peptide bonds in the solution.

UV−vis analysis was performed for the Biuret test solution and that of the pure protein extract using potassium hydroxide and deionized water as basic media. It is known that absorbance is proportional to the concentration of the solution;[46] hence, the UV−vis spectrophotometry analysis was done to confirm the presence of protein. As shown in Table 1, values obtained from the sample analyses indicate high absorbance, which is symbolic to high protein concentrations.

Table 1. Characterization of Protein by the Biuret Test and UV Spectroscopy Analysis

		absorbance			
sample analysis	basic media	300 nm	400 nm	abs. difference (ppm)	color change
biuret test solution under UV−vis analysis	potassium hydroxide	2.518	0.362	2.156	purple
keratin protein under UV−vis analysis	deionized water	0.626	0.070	0.556	

The FT-IR spectral analysis confirmed prominent functional groups including amine (N−H), hydroxy (O−H), and carbonyl (C═O). These are common functional groups present in all amino acids. As shown in Figure 1, these indicate the presence of protein. Amides A and B bands span at 3200−3500 cm^{-1}, amide I band is from 1600 to 1700 cm^{-1}, amide II band is from 1400 to 1490 cm^{-1}, and the region from 500 to 1300 cm^{-1} represents the amides III−VI bands. From the spectrum (Figure 1), it can be seen that IR absorption due to water was minimal; however, broadened amide A and B bands could result from amino acid (amine (N−H) + carboxyl group (COOH)) vibrations. This could be the strongest at the terminal regions of the protein(s) in solution. Water (H_2O) has strong IR absorbance with three prominent bands around 3400 (O−H stretching), 2125 cm^{-1} (water association), and 1645 cm^{-1} (H−O−H bending). The amide I vibration for proteins absorbs between 1600 and 1700 cm^{-1}, overlapping directly with the H_2O bending vibrational band at 1645 cm^{-1}. The intensity of the water absorbance at 1645 cm^{-1} is approximately an order of magnitude higher than the amide I absorbance of proteins. Since water peaks were not intense at around the amide I absorption band, it can be inferred that a high sensitivity was obtained. This is due to the high protein concentration (≥10 mg/mL) in the sample. The amide I band produced a sharp absorption at 1634.82 cm^{-1}. This is characteristic of type I and type II β turns. Traditionally, this is due to the presence of a high percentage of β-sheet in the protein structure. The amide I band (1700−1600 cm^{-1}) is due mainly to the C═O stretching vibration (approximately 80%) of the amide groups coupled with little in-plane N−H bending (<20%).

NMR spectroscopy confirmed the presence of amino acids in the CFP sample. In total, fifteen (15) amino acids were assigned without ambiguity from ^{13}C-NMR data with focus on the carbonyl carbon (C═O) chemical shifts as indicated in Table 2.

Gupta et al. have reported identification of 17 amino acids in a typical chicken feather protein, indicating that two essential amino acids (isoleucine and lysine) were not identified in the CFP sample because their carbonyl signals did not appear in ^{13}C NMR spectra between 169 and 173 ppm, which could be as a result of extremely low quantities.

2.3. Stability Tests (Foaming Experiment/Gelling Test/Emulsifying Activity). *2.3.1. Foaming.* For the foaming experiment, there was a rise of approximately 6% when the mixture was transferred into the graduated measuring cylinder and it was recorded as its new volume. In view of this, the level of foam formed is about 6%. Foams reduce the activity of proteins due to their unreactive nature and hence must not be very high.[4] Thus, it can be considered that for a foaming capacity of approximately 6%, the CFP dispersant has the ability to withstand foams and can therefore form stable emulsions.

2.3.2. Gelling. In this experiment, the CFP dispersant formed a clear yellowish-brown (serum) layer on top of a lower precipitate layer after centrifuging. The precipitate layer completely mixed with the top layer after the dispersant is subjected to a temperature of 90 °C during the gelling test. No gelling behavior was observed as both layers of the dispersant

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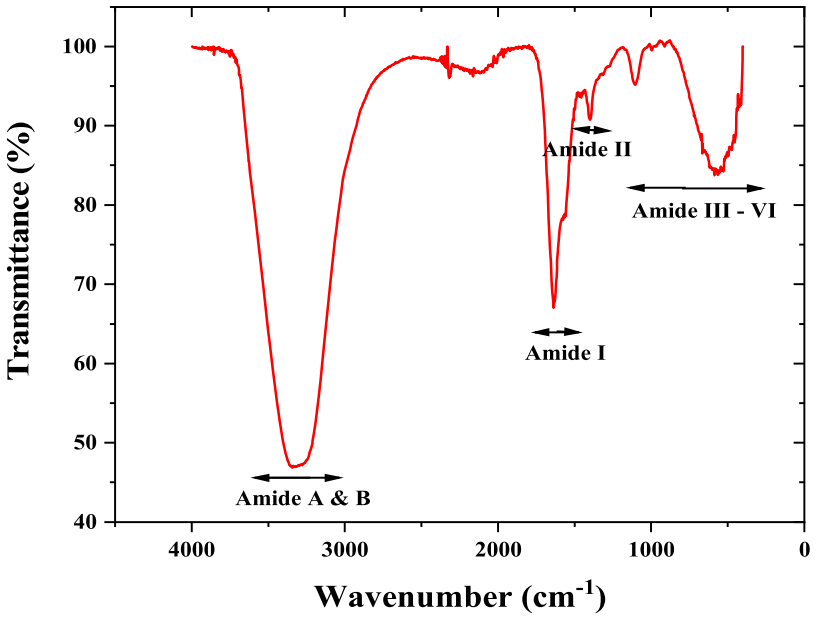

Figure 1. FT-IR spectrum graph of keratin protein.

Table 2. Amino Acids Identified from ^{13}C-NMR Spectra Obtained from Chicken Feather Protein

s/n	δ (C═O,C, ppm)	amino acid	3-letter abbreviation
1	169.71	glycine	Gly
2	170.01	serine	Ser
3	170.10	histidine	His
4	170.33	threonine	Thr
5	170.38	cysteine	Cys
6	170.66	aspartic acid	Asp
7	171.24	phenylalanine	Phe
8	171.31	tyrosine	Tyr
9	171.37	glutamic acid	Glu
10	171.49	valine	Val
11	171.57	arginine	Arg
12	171.68	methionine	Met
13	171.53	alanine	Ala
14	171.57	leucine	Leu
15	173.78	proline	Pro

reappeared at the end of the gelling test. This shows that the CFP dispersant was stable, while the continuous phase was conserved.

2.3.3. Emulsifying Activity. Referring to Figure 2a which shows the control, it can be noticed that emulsions formed without the CFP dispersant initially have a dark-brown coloration, but within a few seconds after removing the shear, there is a complete phase separation that occurs as a result of oil coalescing into larger droplets and creaming occurring at a rapid rate. This means that without the CFP dispersant, the emulsion formed destabilizes rapidly. It can also be deduced from Figure 2b that emulsions formed by adding the CFP dispersant formed dark-brown coloration immediately after removing the shear and stayed stable over a relatively long period of time. It is observed that emulsions formed with the CFP dispersant have a slow coalescing and creaming rate (which will naturally occur over a period due to density difference between the two phases).[49] The average droplet sizes examined before and after creaming are almost of the same value as shown in Figure 3b,d; hence, CFP successfully prevented coalescence. Comparatively, the CFP dispersant-formed o/w emulsions are stable.

Due to the surface hydrophobicity, surface charge, protein size, and molecular flexibility, CFP has the capacity to form stable emulsions. Stable emulsions formed over time as seen in Figure 2b were possible because of the amphiphilic properties of CFP. At the introduction of shear force, CFP migrates, re-aligns appropriately, and adsorbs at the oil−water interface to reduce interfacial tension, resulting in the formation of oil droplets of large surface area, and this is a result of their surface hydrophobicity and charge. During the process of forming homogeneous emulsions, CFP forms strong viscoelastic protective layers around the oil droplets, creating mechanical, electrostatic, and steric barriers to protect the emulsions formed against destabilization. Electrostatic repulsion occurs to keep droplets unattractive to each other, hence preventing droplet conglomeration, and this helps keep the emulsion stable over time. The structure, conformational freedom, and size uniformity of CFP also facilitate steric barrier formation, and this ensures strong anchoring of the hydrophobic head of the dispersant to the oil droplets while ensuring that the hydrophilic tail end of the dispersant is soluble in the surrounding aqueous phase. By steric stabilization, they also provide repulsion between oil droplets by ensuring a sufficient tail length that prevents droplets from coming close enough for van der Waals forces to dominate and force droplet coalescence, thus physically restricting oil droplets from bonding.

2.4. Optical Microscopy of Emulsions and Droplet Size Determination. The stability of emulsions is influenced by the oil droplet sizes obtained after the introduction of mixing energy. The smaller the sizes of the oil droplets, the more stable the emulsions formed. The droplet sizes of the emulsion formed were examined under an optical microscope, and the respective images are shown in Figure 3a,c (showing resistance of the surfactant film at the o/w interface to coalescence of oil droplets). This study was also done to compare the size of droplets before and after creaming. The distribution shown in Figure 3b,d was derived by taking the

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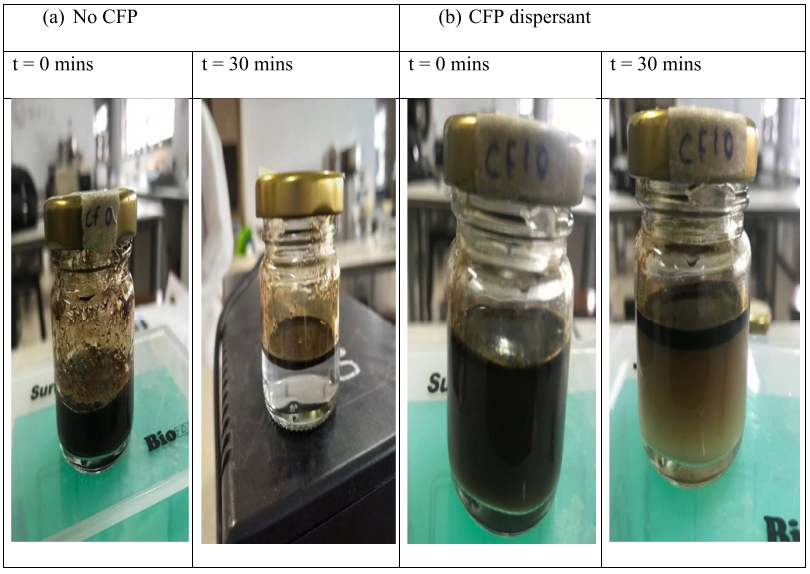

Figure 2. Images of o/w emulsions formed with (a) no CFP dispersant (b) CFP dispersant. Dynamic viscosities of (a) and (b) are of no significant difference (see SI).

optical microscopy images of the emulsion droplets after the zero (0) and 30 min settling time, respectively. The images were taken from 10 different areas of the o/w emulsions, and then, the diameter of 28 randomly selected droplets in each area was measured and used to determine the mean droplet size and the standard error. The mean droplet sizes obtained for Figure 3b,d are 11.4 and 11.9 μm, respectively, and the standard error for both was found to be $\pm$ 0.03 μm. Emulsions of smaller droplet sizes are stable because the droplets have low rising velocities and are unable to form coalescence at the surface. The average droplet sizes obtained before and after creaming are almost of the same value, which indicates that coalescence was almost not possible. The average droplet size obtained after the 30 min settling period confirms that CFP dispersants formed a stable emulsion considering that droplet sizes of <100 μm form relatively stable emulsions.[2]

2.5. Surface Tension and Interfacial Tension. The surface tension was examined for CFP dispersants of concentrations of 200, 400, 600, 800, 1000, and 1200 mg/mL. Using a BZY series automatic surface tension meter, the surface tension of fresh water was measured to be 71.1 mN/m and that of the artificial seawater (35 ppt) was measured to be 71.9 mN/m at 28 °C. The surface tension of the crude oil was also measured and recorded as 27.2 mN/m. A specific amount of crude oil was dispensed onto a known volume of artificial seawater (35 ppt), and its surface tension was measured and recorded as 37.2 mN/m. After the introduction of the CFP dispersant to the oil−water interface, it was found that the dynamic surface tension decreased with the addition of the CFP dispersant. The addition of the CFP dispersant of 200 mg/mL concentration reduced the surface tension from 37.2 to 32.7 mN/m. A further increase in dispersant concentration reduced the surface tension further. As shown in Figure 4, surface tension decreases with increasing CFP dispersant concentration. The critical micelle concentration (CMC) is found to be 980 mg/mL. The reduction in surface tension occurred as a result of protein adsorption at the air−seawater interface.

The interfacial tension was also examined for CFP dispersants of concentrations of 200, 400, 600, 800, 1000, and 1200 mg/mL. A specific amount of crude oil was dispensed onto a known volume of artificial seawater (35 ppt), and its interfacial tension was measured and recorded as 18.6 mN/m, which reduces significantly with the introduction of the CFP dispersant. As shown in Figure 5, it can be seen that the addition of CFP dispersants reduces the interfacial tension of oil−water. The reduction in interfacial tension increases with increasing dispersant concentrations. At 200 mg/mL CFP dispersant concentration, the interfacial tension is 13.6 mN/m, and at 1200 mg/mL, the interfacial tension is 9.4 mN/m.

The CFP dispersant contains both hydrophobic parts and hydrophilic parts that get oriented at the oil−water interface and as a result lowers the interfacial tension. Generally, proteins are slowly adsorbed at the o/w interface due to their high molecular weights, which makes them not so effective in interfacial tension reduction, and this could be the reason for the moderate interfacial tension reduction recorded by CFP. Regardless, there is an indication that CFP dispersants have the capability to reduce the dynamic interfacial tension of the oil−water interface.

2.6. Dispersion Effectiveness of the CFP Dispersant. The dispersion effectiveness (vol %) for the CFP dispersant is determined at different surfactant-to-oil ratios (SOR) at 35 ppt seawater salinity as shown in Figure 6 using the US EPA baffled flask test. The U.S EPA's baffled flask tests is an alternative protocol to their swirling flask test, and it is a laboratory procedure adopted to determine the dispersion effectiveness of the formulated dispersants to be used as oil spill clean-up agents.[50,51]

It was observed that at low surfactant-to-oil ratios, dispersion effectiveness was relatively low but improved at high surfactant-to-oil ratios. The SOR of 200, 400, and 600 mg/mL recorded dispersion effectiveness of 12.35, 30.23, and 42.74 vol %, respectively. However, at higher SORs, 800 and 1000 mg/mL, the dispersion effectiveness was found to be 56.92 and 68.64 vol %, respectively. This indicates that at high SOR, more surfactants are able to diffuse and stay at the oil−water interface to interact with the oil phase and cause a significant reduction in interfacial tension, which results in a relatively higher dispersion effectiveness. Higher dispersion

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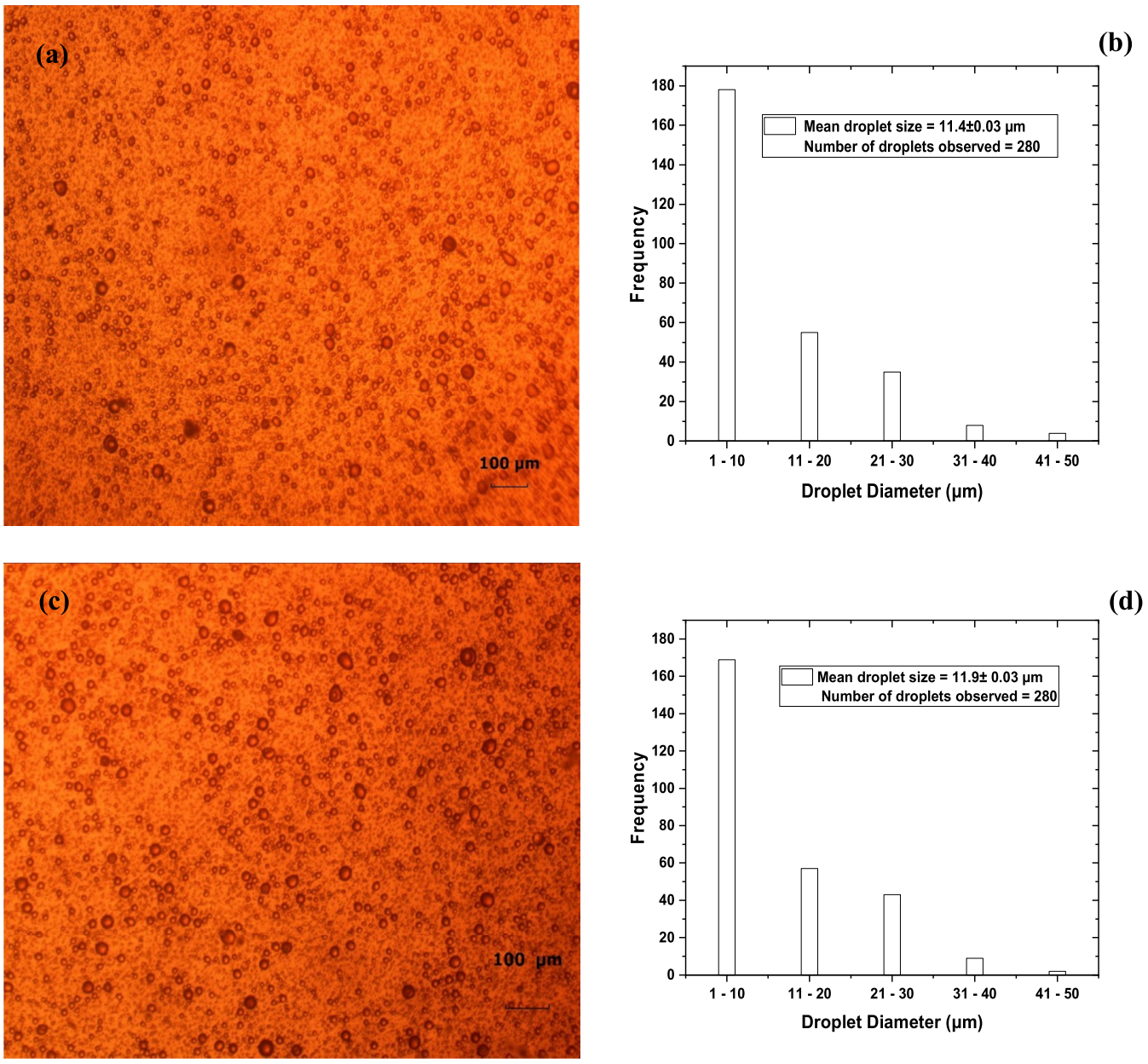

Figure 3. (a) Optical microscopy image of o/w emulsions formed with the CFP dispersant after 0 min. (b) Droplet size distribution of o/w emulsions formed with the CFP dispersant after 0 min. (c) Optical microscopy image of o/w emulsions formed with the CFP dispersant after 30 min. (d) Droplet size distribution of o/w emulsions formed with the CFP dispersant after 30 min.

effectiveness implies that the formulated dispersant is able to clean up oil spills in the marine environment. This means that the dispersant formulated from CFP can be used to disperse crude oil on the surface of water should an oil spill occur, considering the 50 ± 5 vol % dispersion effectiveness in a laboratory test required for a dispersant to be listed onto the US EPA National Contingency Plan (NCP) product schedule.[2,52]

Considering the DE's commercial dispersants, a study conducted by Holder et al. reported that Corexit 9500 which was used during the 2010 Deepwater Horizon incident on the Gulf of Mexico has a dispersion effectiveness of about 82.37 and 75.91 vol % on North star and Terra Nova, respectively (both are light crude oils), in a baffled flask test.[53] Although testing conditions are different and the dispersion effectiveness obtained is lower than that obtained for the mentioned light crude oils, it can be said that the dispersion effectiveness of the CFP dispersant on the Sankofa crude oil, which is also a light crude oil of great promise, and further studies must be conducted to improve upon it.

2.7. Salinity Effects on Oil Dispersion Effectiveness.

Dispersion effectiveness (vol %) determined by using the baffled flask test was used to assess the performance of the CFP dispersant at different seawater salinities (20, 25, 30, 35, and 40 ppt).

As shown in Figure 7, it can be seen that for DOR 2:1, there is an increase in the dispersion effectiveness at 20 ppt when salinity is increased to 25 ppt. A subsequent increase in dispersion effectiveness can be seen at 30 and 35 ppt, but a sudden decrease in dispersion effectiveness is observed at a salinity of 40 ppt. This observation is also consistently seen across DORs of 4:1, 6:1, 8:1, and 10:1. It is known that the

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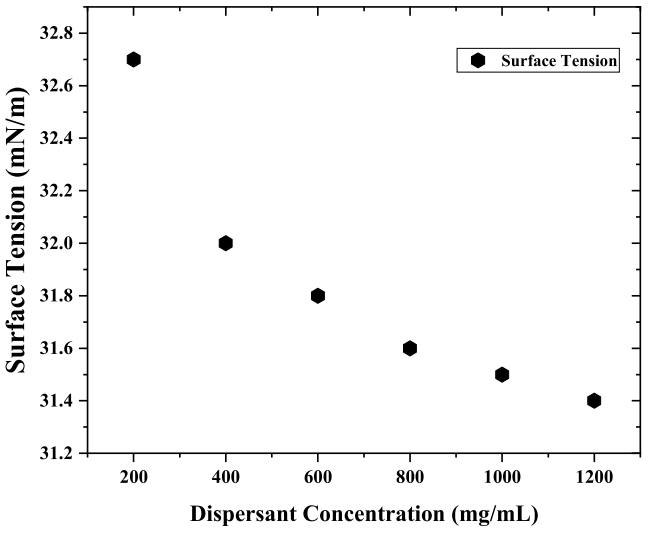

Figure 4. Surface tension measured at different CFP dispersant concentrations. The surface tension was recorded for a period of 30 s.

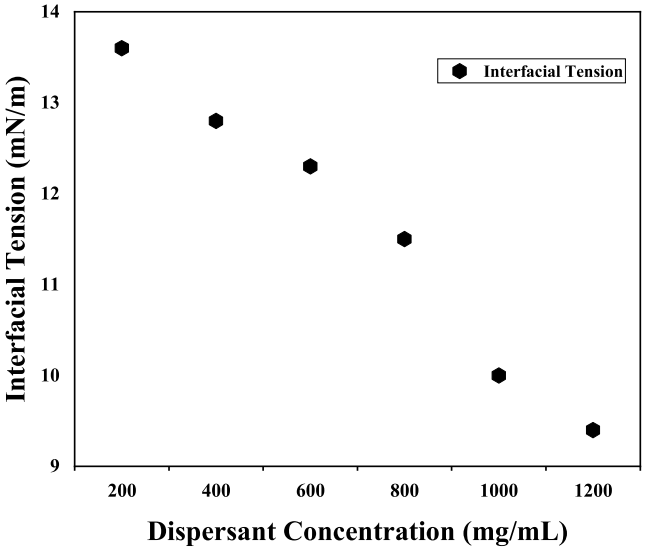

Figure 5. Interfacial tension measured at different CFP dispersant concentrations. The interfacial tension was taken for a period of 5 s.

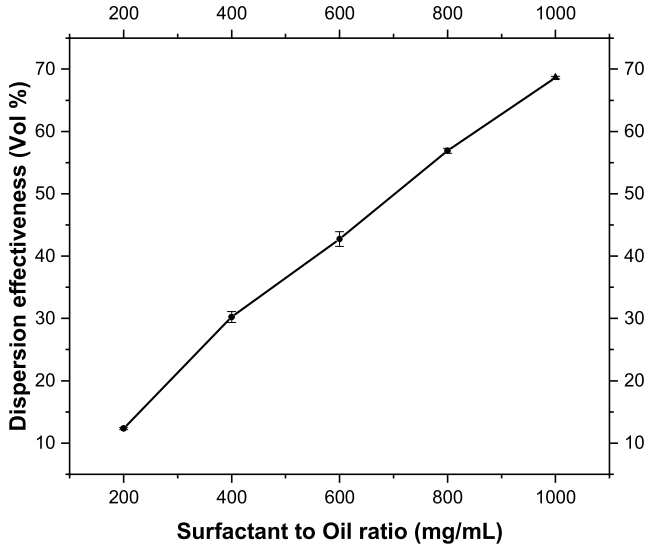

Figure 6. Dispersion effectiveness of the formulated CFP dispersant at different surfactant-to-oil ratios at 35 ppt seawater salinity.

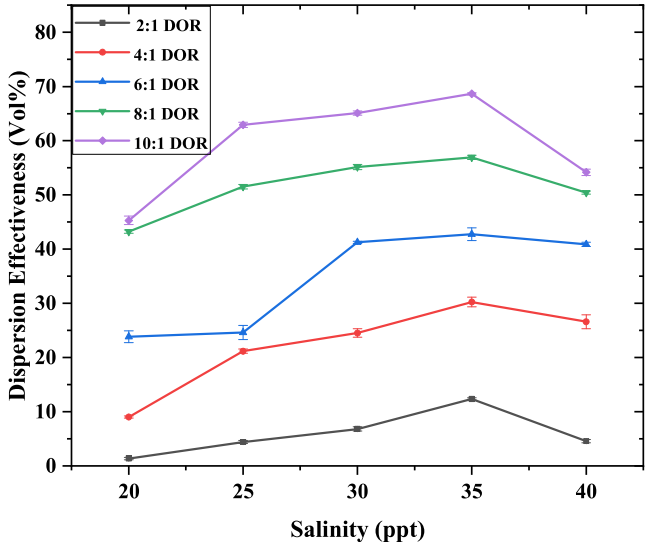

Figure 7. Dispersion effectiveness of different CFP dispersant-to-oil ratios (DOR) at different seawater salinities.

ionic strength of water increases with increasing salinity, and this reduces the solubility of dispersants, while enhancing the surfactant−oil contact rate.[54,55] At increasing water salinities, the hydrocarbon tail in the aqueous phase reduces, which leads to a higher possibility of surfactants staying at the interface to interact with the oil phase, thus resulting in the reduction in interfacial tension, which leads to increasing dispersion effectiveness.[54] Therefore, it can be said that the salting-out effect for the CFP dispersant occurred at increasing salinities of 25 and 30−35 ppt, respectively. However, as observed in Figure 7, dispersion effectiveness for the CFP dispersant decreased at a very high salinity (40 ppt). This is attributed to salting in, which occurs when an increase in the ionic strength of seawater increases the solubility of the CFP dispersants in the aqueous phase. This causes surfactants to diffuse into the aqueous phase rather than staying at the interface to interact with the oil phase. This affects the ability of the CFP dispersant to reduce interfacial tension and hence negatively influences dispersion effectiveness. Taking into account the interfacial tension of the air−fresh water and air−seawater (35 ppt) interfaces, which were measured to be 71.1 and 71.9 mN/m at 28 °C, respectively, it can be deduced that increasing salinity causes an increase in interfacial tension. Therefore, it can be concluded that salinity has an effect on the dispersion effectiveness.

2.8. Fish Acute Toxicity Test of the CFP Dispersant.

The toxicity of CFP was assessed by testing it on Nile tilapia fish, and the results show that the CFP surfactant has an acute fish toxicity of 170.65 mg/L as per the dose−response curve in Figure S2 (SI) obtained from probit analysis of average percentage mortalities from the three CFP replicates (Table S2 in the SI). It is to be noted that there were no mortalities observed in the controls. The LC50 determined from the average percentage mortalities against the logarithmic concentrations as shown in Table S3 (SI) is higher than the maximum dose concentration used in performing the test after the 96 h observation.[56] With an LC50 value of 170.65 mg/L, the CFP surfactant is "practically nontoxic" according to the acute

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toxicity rating scale by the U.S Fish and Wildlife services as shown in Table S1 in the SI and therefore safe to be used for oil spill remediation in the marine environment.

Corexit 9500 and Dispersit SPC 1000 are reported to have an LC50 value of 55 and 10.1 mg/L, respectively.[16] Comparing the LC50 of Corexit 9500, Dispersit SPC 1000 and CFP dispersant indicate that the toxicity of our CFP-formulated dispersant is largely lower than that of some commercially available chemical dispersants.

It can be deduced that the practically nontoxicity of the CFP surfactant is mainly due to the natural composition. Hence, the acute toxicity test results indicate that the CFP dispersant can potentially be used for marine oil spill response knowing that dispersant toxicity on biodiversity is not a concern.

3. CONCLUSIONS

The aim of this study is to formulate a bio-friendly dispersant from protein-rich chicken feather waste, which is capable of crude oil dispersion in the marine environment. The dispersant was formulated from biocompatible and biodegradable proteins extracted from chicken feather, and the solvent used in our formulation is water, which does not possess any harmful substances like the hydrocarbon-based solvents used in traditional commercial chemical dispersant formulations, and hence, it is expected to be environmentally benign and biodegradable. The results obtained in this study show that the dispersant formulated from chicken feather protein has a potential in crude oil dispersion. Emulsions formed by the CFP dispersant were stable over a considerably long period of time, which proves that CFP is a good oil-in-water emulsifier. Emulsion droplet sizes formed over a 30 min period have an average value of 11.9 ± 0.03 μm, which lies well within the less-than-100 μm range of droplet sizes, which suggest that droplets are less likely to re-coalesce and hence formation of stable emulsions.[53] Surface and interfacial tension of the oil−water system were significantly reduced by the addition of CFP dispersants at increasing concentrations. It could be said that at the oil−water interface, CFP adsorbs to reduce the interfacial tension.

To be able to apply CFP dispersants as an oil spill clean-up agent, the dispersant effectiveness was examined using the US EPA's baffled flask test at different surfactant-to-oil ratios. Taking into consideration the baffled flask test using seawater of 35 ppt salinity, dispersion effectiveness values recorded at 200, 400, 600, 800, 1000 mg/mL surfactant-to-oil ratios were 12.35, 30.23, 42.74, 56.92, and 68.64 vol %, respectively. Dispersion effectiveness increased with increasing surfactant concentration. Salinity studies conducted on the CFP dispersant indicated that salinity has influence on the dispersion effectiveness of the dispersant formulated.

Furthermore, the CFP formulation gave an LC50 value greater than 100 mg/L after 96 h of exposure.

In conclusion, a safe and effective novel biodispersant has been formulated from chicken feather protein. In addition, a smaller dispersant-to-oil ratio will increase the potential of the product to replace the existing hydrocarbon-based dispersants; hence, further studies will be conducted to improve upon the dispersant-to-oil ratio. Future studies such as combining CFP with other emulsifiers will also be considered to improve upon the surface and interfacial activities of the dispersant as well as enhancing its dispersion effectiveness.

4. EXPERIMENTAL SECTION

4.1. Materials. Chicken feathers were obtained from nearby slaughter houses in Madina market, Accra. All chemicals were analytical grade and used without further purification. Ether was purchased from Daejung chemicals; the following chemicals were obtained from VWR chemicals: sodium sulfide, dichloromethane (DCM), and ammonium sulfate. Sodium hydroxide and potassium hydroxide were obtained from Merck, thioglycollic acid was obtained from Paskem Fine Chem Private limited, hydrochloric acid (HCl) was obtained from Fisher chemical, copper(II) sulfate was obtained from Breckland scientific supplies, Instant Ocean salt was obtained from Spectrum Brands Pet LLC (Blacksburg, VA) and deionized water. Light crude oil was obtained from the Sankofa oil field (properties of the crude oil used in this study were determined; density of 849.3 kg/m^3 at 15.6 °C, kinematic viscosity of 4.658 cSt, measured @40 °C, and API gravity of 35.04°).

4.2. Extraction of Protein. A method similar to that of Gupta et al.[46] was adopted for this procedure. The various methods under this procedure which covers pre-treatment of feathers, dissolving chicken feathers, preparation of ammonium sulfate solution, protein precipitation, and purification are detailed in the Supporting information (SI).

4.3. Characterization of the Protein sample. To confirm the presence of protein in the sample extracted, it was characterized by the biuret and absorbance tests, Fourier transform infrared spectroscopy (FT-IR) analysis, and nuclear magnetic resonance (NMR) analysis. Characterization methods are detailed in the SI.

4.4. CFP Dispersant Formulation. 10 g of CFP is measured into a 100 mL volumetric flask and topped up with deionized water to the 100 mL mark. The mixture is stirred at 200 rpm at 25 °C on VWR hotplate/stirrer (model: 984VW4CHSUKA) equipment for 10 min to make a homogeneous mixture of 10% wt/v. Drops of HCl are added to adjust the pH to neutral. The 10% wt/v (0.1 g/mL) concentration of the dispersant formed is excessively low compared to the 83% surfactant concentration of Corexit dispersants.[57] 0.1 g/mL CFP was prepared to test the efficiency of the dispersant at low concentrations. The potency of the CFP dispersant was measured by its ability to form stable emulsions over a period, droplet sizes, surface, and interfacial activities and the dispersion effectiveness(DE) using the US EPA's baffled flask test (BFT).

4.5. Artificial Seawater Preparation. 40, 35, 30, 25, and 20 g/L which represent 40, 35, 30, 25, and 20 ppt concentrations of artificial seawater are prepared, respectively, by dissolving appropriate amounts of Instant Ocean salt in 1 L of deionized water in a volumetric flask. The respective solutions are magnetically stirred on a Cole-Parmer Stable-Temp stirrer at 250 rpm for 24 h to ensure a homogeneous mixture. The pH of the various artificial seawaters prepared is 7.8.

4.6. Stability Tests (Foaming Experiment/Gelling Test/Emulsifying Activity). *4.6.1. Foaming Experiment.* 3 g of CFP dispersant is measured into a 250 mL beaker, and an amount of deionized water is added to make the total volume of mixture 100 mL in the beaker. The mixture was shaken and whipped in a blender for 30 s. After whipping, the mixture is poured in a 200 mL-graduated measuring cylinder, and the new volume is recorded.

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4.6.2. Gelling Test. A known amount of the dispersant is first centrifuged at 6000 rpm for 10 mins to observe its phase separation. An amount of CFP dispersant is kept in a water bath (Clifton Unstirred bath) at 90 °C for 30 min and is stored in a refrigerator at 6−8 °C for 24 h to obtain gels. The gels formed are heated at 90 °C for 10 min to determine its stability. The precipitates formed after the 2nd heating are observed and recorded.

4.6.3. Emulsifying Activity. To examine the stability of emulsions formed by the CFP dispersant, 1 mL of crude oil is added to 10 mL of artificial seawater of normal seawater salinity (35 ppt). A known quantity of CFP dispersant is added to the 1:10 crude oil−artificial seawater. The mixture is vortexed at 2500 rpm for 2.5 min using a Stuart vortex mixer (CAT no. SA8). The stability of emulsions over a period of time is observed, and images of the emulsion are taken. After the exertion of the shear, the emulsion is made to settle for a 30 min period, while pictures are taken at 0 and 30 mins to examine the stability of the o/w emulsions formed. The same process is repeated for 1:10 v/v crude oil−artificial seawater without the addition of the CFP dispersant as a control. The dynamic viscosity of emulsions formed is measured using a Cole-Parmer viscometer (SN: VCPR140021) as detailed in the SI.

4.7. Optical Microscopy of Emulsions and Droplet Size Determination. 1 mL of crude oil is added to 10 mL of artificial seawater (35 ppt). An appropriate amount of CFP dispersant is added to the 1:10 crude oil−artificial seawater. The mixture is vortexed at 2500 rpm for 2.5 min using a Stuart vortex mixer (CAT no. SA8). Optical microscopy images are taken using an AmScope FMA050 after zero (0) and 30 min of settling.

4.8. Surface and Interfacial Tension Determination. The reduction in interfacial and surface tension of the crude oil-in-saline water (35 ppt) emulsions is estimated by the Wilhelmy plate method (platinum plate method). A BZY series automatic surface tension meter (model: BZY-101) is used for checking the surface and interfacial activities of the CFP dispersant by following the respective protocols of the equipment as detailed in the SI. The test was carried out under ambient room conditions.

4.9. Dispersion Effectiveness Using the Baffled Flask Test. A method similar to that of Venosa et al.[50] was adopted for this study to determine the dispersion effectiveness of the CFP dispersant. 120 mL of artificial seawater is measured into a 250 mL baffled flask.100 μL (0.1 mL) of crude oil is carefully added directly onto the surface of the artificial seawater in the 250 mL baffled flask using a 100−1000 μL Pipet4u micropipette with a 1 mL pipette tip attachment. 200 μL (0.2 mL) of the 100 mg/mL CFP dispersant is added to the baffled flask (this is repeated for 400 μL (0.4 mL), 600 μL (0.6 mL), 800 μL (0.8 mL), and 1000 μL (1 mL) to obtain a surfactant-to-oil ratio of 200, 400, 600, 800, and 1000 mg/mL, respectively). It is carefully done to ensure that the dispersant touched the oil without first contacting the water. The baffled flask is shaken for 10 min at 200 rpm using a VWR advanced digital 3500 shaker. After the 10 min of shaking, the flask is allowed to settle for 10 mins. After the 10 min settling time, 2 mL of the sample is drained from the stop cork and discarded. A volume of 30 mL of the oil-in-water emulsion sample formed is collected using a 100 mL-graduated cylinder. The 30 mL sample collected is transferred into a 250 mL separation funnel and extracted two times using 10 mL of fresh DCM, making the total volume of DCM 20 mL. The dispersed oil in the extracted sample is estimated using UV−vis spectroscopy at an absorbance difference of 300−400 nm. The baffled flask test is performed for all seawaters of various salinities in this study. The baffled flask test was conducted in triplicate.

4.10. Fish Acute Toxicity Test. The CFP surfactant was tested on Nile tilapia (Oreochromis niloticus) to determine its acute toxicity by measuring the lethal effect after a 96 h exposure in a static test. The test procedure as shown in Figure S1 was centered on the OECD Guideline No.203 (OECD, 2019)[58] and was used as the standard method in performing every test. An initial limit test (100 mg/L) was performed to check the mortality at the LC50 value. The definitive tests were conducted at different concentrations of 25, 50, 75, 100, and 125 mg/L to demonstrate that the LC50 value is greater than the limit test concentration. The lethal concentration at 50% (LC50) was computed using probit analysis based on the observations and response after the 96 h exposure.[56,59] Acute toxicity tests were performed in three replicates as shown in the Supporting Information (Table S2). The details of the toxicity analysis of the chicken feather protein dispersant are presented in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c04417.

Additional experimental details covering the chicken feather protein extraction procedure; characterizing of the product; protein hydrolysis procedure; surface and interfacial tension procedure; measurement of emulsion viscosity, and acute toxicity assessment of CFP (PDF)

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Notes

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