HYPERBRANCHED POLYMERS AS BIOCOMPATIBLE OIL DISPERSANTS: INFLUENCE OF SALINITY, PH, AND CONCENTRATION ON DISPERSION EFFECTIVENESS

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Environmental Engineering and Earth Sciences

by
Kristen Mae Carpenter
August 2016

Accepted by:
Dr. David Ladner, Committee Chair
Dr. Kevin Finneran
Dr. David Freedman

ABSTRACT

The Deepwater Horizon oil spill in 2010 marked one of the biggest spills in history. Like many other oil spills, Corexit 9500 was used to attempt to prevent some of the environmental impacts of the spill. The abundant use of Corexit during this spill raised public awareness and called into question the dispersant's toxicity and long-term impacts on the environment. These concerns brought to light the need to discover less toxic, more biodegradable oil dispersants. This research helps determine the feasibility of using hyperbranched polymers (HBP) as oil dispersants by comparing their oil dispersion effectiveness to that of the commonly used dispersant, Corexit 9500.

Hyperbranched polyethylenimine (Hy-PEI) polymers of various molecular weights were the first potential dispersants tested. In artificial seawater with a 0.02 (1:50) dispersant-to-oil ratio (DOR) Hy-PEI polymers with low molecular weights (1.2 and 1.8 kiloDaltons [kDa]) showed very little dispersion capability. The higher molecular weights (10, 70, and 750 kDa) showed better dispersion potential; however, only 10 kDa had an oil dispersion efficiency (82.9%) as high as Corexit (72.9%).

The effectiveness of the Hy-PEI polymers and Corexit was also determined for a range of DOR's. For each polymer and Corexit the DOR was varied from .0015 to 1. The goal was to observe how increasing the amount of dispersant applied to the oil affected how well the oil was dispersed. With increasing DOR , 1.2 and 1.8 kDa had a slight increase in dispersion effectiveness. Both Corexit and 10 kDa showed an increase in dispersion effectiveness as DOR increased. However, 70 and 750 kDa appeared to have a maximum dispersion effectiveness at a 0.02 DOR, followed by a decrease in effectiveness as their DOR

was increased. It is likely that as the DOR of the larger polymers were increased passed 0.02, they began to interact more with each other and resulted in an agglomeration of dispersant that decreased their ability to keep the small oil droplets dispersed.

The effectiveness of the Hy-PEI polymers and Corexit was also determined for various aquatic environments. In order to stimulate different environments that could be exposed to oil spills, the salinity and pH of the artificial seawater used in the dispersion tests was altered. Artificial seawater was diluted with DI water in order to achieve saltwater with 0, 10, and 35% of the original seawater salt concentration. The clearest trends were seen with Corexit, 70 kDa, and 750 kDa. Corexit showed an increase in effectiveness as salt concentration increased. This is due to Corexit being designed with surfactants that are tailored to higher salinities. In freshwater, the surfactants in Corexit are altered to promote water-in-oil emulsions, this prevents the surfactants from adequately stabilizing the oil droplets in the water column. However, with the 70 and 750 kDa Hy-PEI polymers, there appeared to be a drop in dispersion effectiveness at the highest salinities. It is possible that high ionic strength increased the aggregation of the polymers with high molecular weights.

In addition to the Hy-PEI polymers, various HBP's with a polyethylene glycol core and polyester links (Hy-PE-PEG polymers) were also tested for dispersion capabilities. These were chosen because their hyperbranched structure is made up of polyester linkages. Polyester linkages have been shown to have higher biodegradability than PEI, making the Hy-PE-PEG polymers potentially more appropriate for environmental remediation. Also, their neutral end groups are less toxic to aquatic organisms than the positively charged end group of the Hy-PEI polymers. However, the lack of positively

charged end groups also appeared to decrease dispersion effectiveness; none of the Hy-PE-PEG polymers demonstrated a dispersion effectiveness as high as that of Corexit.

DEDICATION

I dedicate this thesis to my teachers from Kindergarten to Clemson, all who showed me that learning is a fun, never-ending process and knowledge is a powerful tool. I also dedicate this to my family who has never ceased to support or encourage me along the way.

ACKNOWLEDGEMENTS

I must first acknowledge my advisor, Dr. David Ladner, who was willing to provide academic and research guidance at a minimum of once a week. I would also like to thank the remainder of my committee, Dr. David Freedman and Dr. Kevin Finneran. All three professors were not only willing to help with my graduate thesis, but also helped guide me through my undergraduate and graduate career at Clemson. Without them I would not have discovered my enjoyment of Environmental Engineering or have become the engineer I am today.

In addition, I need to acknowledge Ying Tu and Kimberley Bourne, both of whom helped me run various experiments. Lastly, the funding for this project was provided through the Environmental Protection Agency, Grant RD835182.

TABLE OF CONTENTS

Page

Table of Contents (continued)

Page

Table of Contents (continued)

Page

LIST OF TABLES

LIST OF FIGURES

List of Figures (continued)

Page

List of Figures (continued)

Page

List of Figures (continued)

Page

List of Figures (continued)

Page

LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviations

BFT	Baffled Flask Test
BP	BP Petroleum Industry Company
DCM	Dichloromethane
DDI	Distilled Deionized
DLVO	Derjaguin, Landau, Verwey, and Overbeek
DOR	Dispersant to Oil Ratio
DSA	Drop Shape Analysis
EPA	Environmental Protection Agency
HBP	Hyperbranched Polymer
HLB	Hydrophlic Lipophile Balance
Hy-PE-PEG	Hyperbrachned Polyester Polyethylene glycol
Hy-PEI	Hyperbranched Polyethylenimine
IFT	Interfacial Tension
LSC	Louisiana Sweet Crude
MW	Molecular Weight
OWR	Oil to Water Ratio
PAHs	Polycyclic Aromatic Hydrocarbons
PAMAM	Polyamidoamine
PEG	Polyethylene Glycol
ppt	parts per thousand
SOP	Standard Operating Procedure

1 INTRODUCTION

Since the discovery of subsurface petroleum hydrocarbons – what we often simply refer to as “oil” – its use has been continuously increasing, as has its presence in the world’s oceans. Large oil spills account for less than 10% of the oil release into the oceans [1]; however, all oil spills are responsible for the release of 37 million gallons of oil per year worldwide [2]. Due to the sudden release of extreme volumes of oil, these spills have numerous negative environmental impacts. A common method to help minimize the impact of oil spills is the use of oil dispersants.

In recent history, the most well-known oil spill is the Deepwater Horizon disaster that occurred in the Gulf of Mexico in April 2010. In order to combat the effects of this spill, the chemical dispersant Corexit 9500 was abundantly used. A total of 1.4 million gallons of Corexit were added to the ocean surface with an additional 0.77 million gallons added directly into the deep-water leak [3]. Before application, many questions of Corexit’s long-term environmental effects and toxicity were unanswered. Recent research has called into question the benefits of applying Corexit and if they outweigh its detrimental effects [4]. Since dispersants remain to be one of the best available technologies for negating various environmental impacts [5], there is a need for well-researched dispersants that have been proven to be effective, more biodegradable, and less toxic.

Dispersants break down oil droplets small enough to allow naturally occurring hydrocarbon-degrading microbes to more readily access and degrade the oil. The dispersion can prevent many environmental problems and the resulting increased bioavailability accelerates oil degradation [5]. However, one of the main controversies of dispersants is that they result in an environmental trade-off; their application reduces oil exposure to surface organisms, intertidal species, and protects shores, but dispersants increase exposure to aquatic life found in the water column [6].

In addition to this controversy, the chemicals and other ingredients present in Corexit have been called into question in regards to their potentially hazardous presence in ocean environments. To continue the search for less toxic, more biodegradable dispersants, this project examined the effectiveness of using polymers as oil dispersants in a variety of water types. These polymers have multiple properties that allow them to not only be efficient dispersants, but also have the potential to be designed with less toxic, more biodegradable characteristics when compared to the chemical dispersant, Corexit.

2 BACKGROUND

2.1 Oil Spills

The world's population is heavily reliant on oil, and this high demand pushes for the continuous pumping and transport of vast amounts of oil. Due to its large presence in the world, oil spills are an unfortunate, but inevitable reality. Since 1974, 9,351 accidental oil spills have been recorded [7] and it is estimated that at least 37 million gallons of oil are released via spills worldwide every year [2].

Crude oil is a combination of many hydrocarbons that contain anywhere from four to sixty carbon atoms [8]. There are many types of crude oil, but they all contain various amounts of carbon, hydrogen, sulfur, nitrogen, oxygen, minerals, and salts [8].

Once oil is released into the environment, the different components of the oil partition with various environmental compartments [8]. Oil spill contamination can have detrimental effects on the entire ecosystem that has been exposed to the oil due to its widespread nature and the bioaccumulation of petroleum hydrocarbons. Salt marshes, wetlands, swamps, and seabirds are especially susceptible to damage from oil spills, a damage that can last for decades [9].

Though they only account for a small percentage of oil presence in the oceans overall, oil spills are an extreme hazard to the environment. Aquatic life in both the ocean and freshwater environments is sensitive to the harmful components of crude oil; as well as birds and mammals that are affected from oil attachment and oil ingestion [8]. In 1995 there were 5,000 tonnes of oil spilled into the ocean as a whole; the next year the *Sea Empress* accounted for the release of over 5,000 tonnes of oil in just one spill. *Sea Empress* demonstrates just one example of the magnitude of oil that can be released into the ocean from a single spill [10]. A detrimental spill in the wrong place at the wrong time has the potential to cause irreparable consequences to the aquatic environment and its species [9].

2.1.1 Deepwater Horizon

April 20, 2010 marked the beginning of the largest marine spill in recorded history. An explosion on The Deepwater Horizon oil rig, operated by the oil and gas company British Petroleum (BP), caused a fracture in the core that allowed oil to be released into the Gulf of Mexico [11]. The oil continued to leak into the gulf at alarming rates for over 80 days, pouring an estimated 4.4 million barrels [3] or almost 206 million gallons of light crude oil into the gulf [12].

This spill had major impacts on both the environment and the economy on the Gulf of Mexico. The economy of this region relies heavily on fishing and tourism. As a result of the spill, many fisheries were closed due to contamination concerns, and tourists were hesitant to visit potentially polluted beaches [11]. The monetary damages done to BP, the environment, and the gulf coast economy have been estimated at almost $40 billion [13].

Environmentally, eight national parks and four national wildlife refuges were threatened by exposure to the oil spill [14]. These and other areas being effected put bluefin tuna, sea turtles, sharks, whales, porpoises, dolphins, brown pelicans, oysters, shrimp, blue crap, marsh fish, shorebirds, and songbirds at risk [15]. From the start of the spill until August 16, 2010 over 7,000 birds, sea turtles, and dolphins were found dead or injured, half of which had visible oil present on their bodies. These large numbers do not account for many of the small birds and fish that were also harmed by the oil spill [16]. The EPA stated that the Deepwater Horizon spill could be “one of the greatest environmental challenges of our time” [17].

2.1.2 Oil Spill Recovery

An ecosystem is labeled as recovered when its biological community of plants, animals, and microorganisms begin functioning normally. In the case of an oil spill, the organisms would exhibit the same behavior as they had prior to the spill. An ecosystem can take several decades to fully recover from a large oil spill [9].

The main natural processes that help break down oil spills are weathering, dilution, diffusion, and dispersion [3]. When oil is spilled into the ocean it initially forms a layer of oil, several millimeters in thickness, that spreads over the ocean's surface. As the oil slick spreads its volatile components quickly evaporate. Then the oil slick is exposed to weathering and wave motion and begins to break up into oil droplets that descend into the water column. Once oil droplets are small enough they will stay suspended in the water column and naturally occurring bacteria can begin to degrade them [9]. The microbial degradation converts the dispersed oil into biomass, water, and carbon dioxide [18], as shown in **Figure 2-1**. Almost all types of hydrocarbons that make up natural oil can undergo microbial oxidation, but the speed of this process is dependent on the type of oil, the aquatic environment (salinity, pH, temperature, etc.), weathering that the slick is exposed to, and other factors [8]. Various types of oil can possess a biodegradability ranging from 70-90% [19].

There are over a 170 species of bacteria, yeasts, and fungi that have the ability to oxidize hydrocarbons in various environments [20]. Many of these oil-degrading microorganisms occur naturally in oceans [10]. Ecosystems can begin to biologically recover from oil spills once a microbial community can overcome the toxic properties remaining in the dispersed oil [9]. How quickly and well an oil spill is dispersed is an important factor in how much an oil spill will impact the marine ecosystem; the faster the oil is degraded, the more limited its adverse effects become. However, natural dispersion happens slowly and the naturally occurring oil-degrading microorganisms are limited by the oil's surface area [21]. This not only limits the rate at which oil degradation can occur, but also the percentage of the oil that will be dispersed and degraded [22].

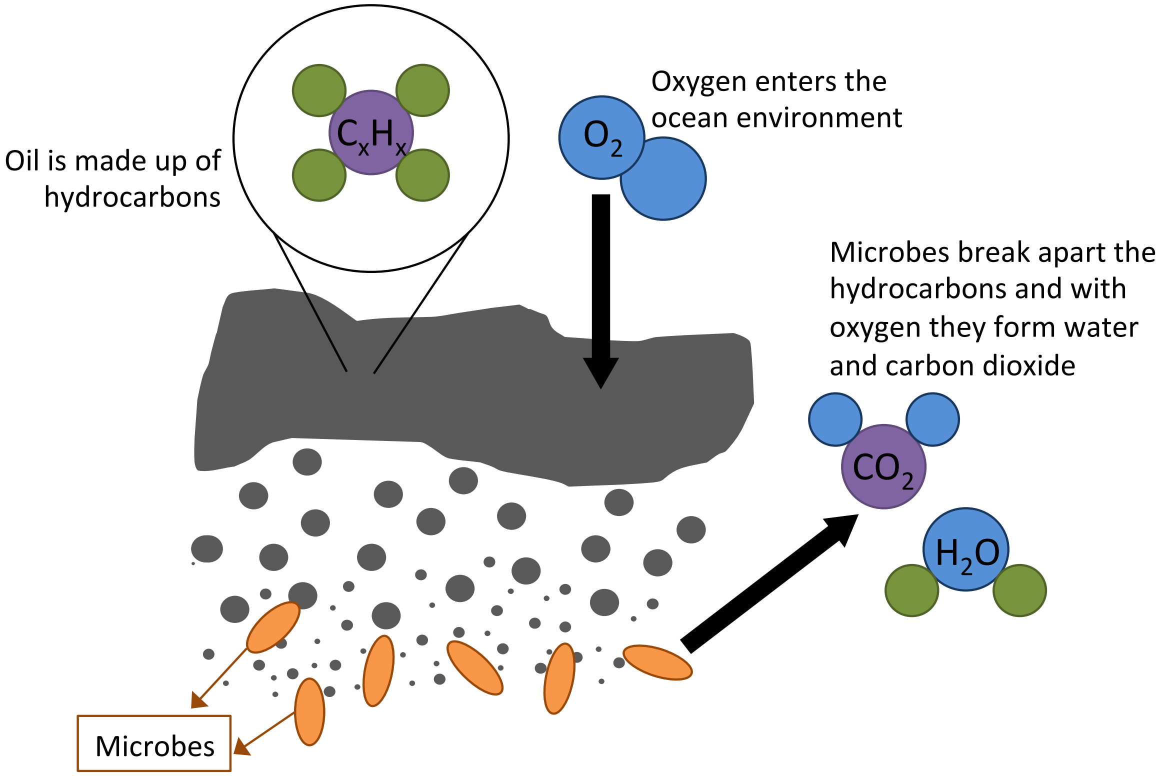

Figure 2-1. A schematic showing the process of oil degradation [23].

2.2 Dispersants

Since dispersion is believed to be the main natural cause of oil removal from the ocean's surface, oil dispersants were designed to accelerate this process. Dispersants are able to break up the oil slick faster and make the oil more readily available for the microbes to degrade it. Since dispersants make oil more bioavailable, microbial oxidation of the oil is enhanced and the natural oil degradation process is accelerated [9, 24] [24].

.

The 1993 MB *Braer* spill off the coast of Scotland, demonstrated how an oil spill with no oil slick would have significantly fewer seashore effects. During and after the *Braer* oil spill the oil was exposed to extreme storms, which effectively dispersed the oil through waves, evaporation, and dilution. Due to this, a large oil slick did not form. This dispersion limited the seashore's exposure to the oil and allowed for a much faster recovery because the majority of the oil was dispersed into the sea [25]. Dispersants build upon this model that if an oil slick can be broken up, environmental recovery can be significantly accelerated [9]. Whereas oil that reaches shorelines has an environmental residence time of years, dispersed oil has a fraction of this half-life, on the magnitude of weeks [26].

In ideal conditions, where the oil becomes dispersed enough, the microbial oxidation of oil can begin to occur in as little as days [27] and a high majority of the oil will be completely biodegraded within weeks to months [26]. However, if adequate dispersion does not take place, the degradation of oil can take significantly longer. In addition, the oil could solidify and form tar balls, which are much more highly resistant to microbial breakdown [27]. Dispersants are employed to help insure that the oil is dispersed adequately.

Dispersants are common and have been used in several oil spills, including Deepwater Horizon. However, there is controversy surrounding oil dispersants because they pose an environmental trade-off. Dispersants are able to reduce the exposure of oil to surface organisms, intertidal species, wetlands, and shorelines [24]; however, they increase exposure to aquatic life found in deep water [17]. Currently, dispersants remain to be the best available technology for mitigating many of the detrimental effects of oil spills [5] and in most cases the environmental concerns of applying dispersants are outweighed by the environmental benefits [26].

2.2.1 Chemical Dispersants

Chemical dispersants are made up of surfactants that are dissolved or suspended in a solvent [28]. The surfactants stabilize oil droplets in water by decreasing the surface tension between the oil and water interface. This helps prevent the oil droplets from coalescing and rising back out of the water column. Surfactants are made up of a hydrophilic (water-loving) head and a lipophilic (oil-loving) tail; the ratio of these different components is called the hydrophilic-lipophilic balance (HLB), which is used to characterize surfactants. Lower HLB's (1-8) promote water-in-oil emulsions whereas higher HLB's (12-20) promote oil-in-water emulsions. Oil dispersants generally have a mid HLB range of 8-12. This middle range can form both types of emulsions, but more often promote oil-in-water emulsions [29]. The surfactant mechanism for oil dispersion in shown in **Figure 2-2**.

One of the most commonly employed chemical dispersants is Corexit. Corexit was first developed in the 1960's and is currently produced by Nalco. The newest chemical formulation of Corexit, Corexit 9500, is reported to have less toxic ingredients and additional biodegradable components to encourage microbial growth [10]. A table of Corexit 9500's six major ingredients, as reported by Nalco, is shown in **Table 2-1**.

Since its development, Corexit has been used as a dispersant in numerous oil spills. In the Deepwater Horizon spill Corexit was not only used on the surface of the oil slick, but was also injected underwater directly into the leaking oil stream; this was the first time Corexit was applied in deep water. Even though the environmental effects of Corexit were largely unknown, especially with subsea application, 1.4 million gallons of Corexit were applied at the surface and 771,000 gallons were applied to the oil stream underwater [12].

The abundant and unprecedented use of Corexit in the Deepwater Horizon spill significantly raised public awareness of Corexit in regards to its effectiveness, toxicity, and the lack of knowledge and research regarding the dispersant. Even though Corexit has improved upon its original makeup, it has still been found to be more toxic than crude oil alone [4, 29, 30] [4] [30] [31] and has also been shown to have cytotoxicity on various organisms that live in the ocean [31-32] [32] [33]. Ongoing research is still being conducted to further determine Corexit's toxicity, effectiveness, and fate in the Gulf of Mexico. Corexit has continued to develop a negative perception of chemical dispersants, and has caused an increase in research to find less toxic, more biodegradable oil dispersants.

Table 2-1. Table listing the reported ingredients of Corexit (produced by Nalco) and their common uses. [34]

CAS #	Name as listed by Nalco	Common Names	General Uses	Used In*
1338-43-8	Sorbitan, mono-(9Z)-9-octadecenoate (SPAN 80)	Sorbitan Monooleate, Span 80	Emulsifier for foods and cosmetic products	DermaQuest Solar Moisturizer SPF 30***
9005-65-6	Sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs.	Polysorbate 80, Tween 80, Ethoxylated sorbitan monooleate, Polyoxyethylene Sorbitan Monooleate**	Emulsifier, commercial stabilizer, flavoring ingredient, color diluent, and defoaming agent used in foods, vitamins, medicines, and vaccines	Fiber One and Kroger brand cottage cheese, Vlasic jarred pickles, Heinz Premium Genuine Dills, Fudgesicle Fudge Pops, Several Weight Watchers ice cream bars, Blue Bunny Orange Dream Bar
9005-70-3	Sorbitan, tri-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs	Tween 85, Polyethylene glycol sorbitan trioleate, Polyoxyethylene sorbitan trioleate, Polysorbate 85	Emulsifier in medicines, stabilizer for color materials and emulsion polymerization, adjuvant of agrochemicals****, ingredient for water based metal process cutting fluids, surface coating type for antistatic agents	***
577-11-7	Butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (1:1)	DSS, DOSS, AOT, dioctyl sodium sulfosuccinate**	medication for laxatives, agrochemical adjuvant, ingredient in tick and flea medication, emulsifier in foods	Fanta, Hawaiian Punch low calorie drink mix, various Flavor Aid drink mixes, De Flea Pet and Bedding Spray
29911-28-2	Propanol, 1-(2-butoxy-1-methylethoxy)	isopropyl alcohol, dipropylene glycol	chemical reactant as a chemical intermediate, coalescing agent, and coupling agent	Rubbing alcohol, Hand sanitizer
64742-47-8	Distillates (petroleum), hydrotreated light	Hydrotreated kerosene, paint thinner	ingredient in pesticides, larvicides, car wax, car tire protectants, car fuel system cleaners, and camp fuel	Coleman Camp Fuel, Goo Gone Liquid

*This is a selection of examples of areas where these ingredients are used. It is not a comprehensive listing of every product that utilizes these substances.
**These common names are the most likely way these compounds will be listed in foods.
*** Limited information available online
****Adjuvant of Agrochemicals- used in various chemical mixtures, specifically used in the agricultural industry, in order to increase the effectiveness of the chemicals

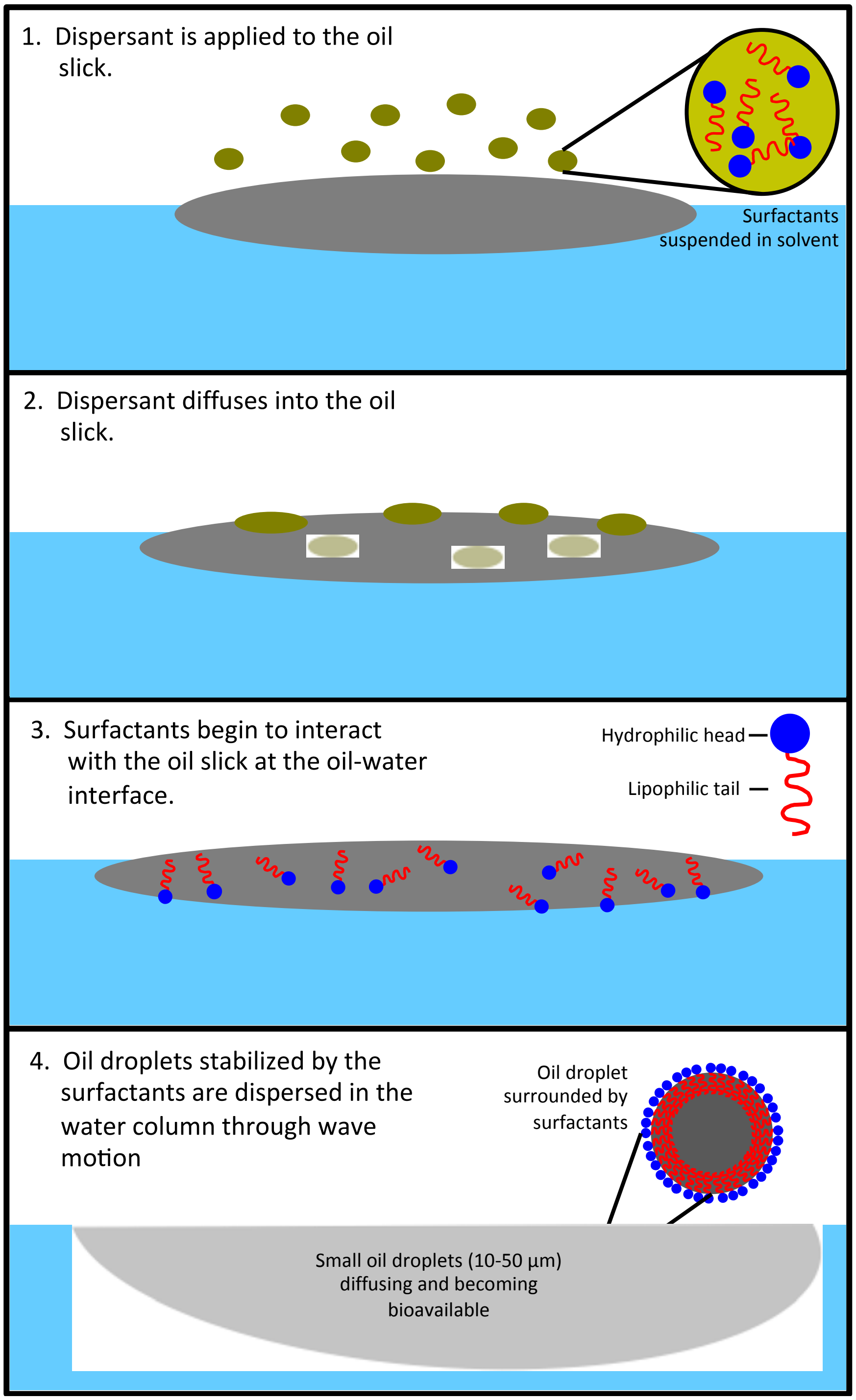

Figure 2-2. Schematic demonstrating how dispersants break up oil slicks into dispersed oil droplets [35].

2.2.2 Novel Dispersants

In order to meet the desire for more environmentally benign dispersants, a vast amount of research has been done to try and find dispersants with a lower toxicity and higher biocompatibility and biodegradability. Toxicity is defined as harm towards cells, organs, or a multi-organ system [36], biocompatibility is when a material is able to be present in an environment without causing harm to living organisms, and a material is biodegradable when it can be decomposed by microorganisms [37]. A variety of materials have been proposed and studied to meet these desires, including the addition of nutrients and/or microbial communities, nanoparticles, dendrimers, and other polymers.

In an attempt to stimulate additional growth of oil-degrading microbes, fertilizers containing nitrogen and phosphorous have been added to oil spills. These two nutrients are probably the most limiting nutrients to microbial growth in environments exposed to an oil spill [10]. Fertilizer addition was first attempted in the 1989 *Exxon Valdez* spill [38]. Two fertilizers containing nitrogen and phosphorous were added to both the oil slick surface and subsurface [39]. Results showed that the addition of these nutrients did stimulate microbial activity, which in turn accelerated the oil's biodegradation by a factor of 2-5. In addition, there was no evidence that the application of the fertilizers caused an adverse impact to the environment [40]. Though proposed, there has been less evidence that adding a microbial inoculant to an oil slick will increase biodegradation rates faster than those of the naturally present microorganisms, but the potential is being further investigated [41].

Nanoparticles have been proposed as an option for novel oil dispersants. Guilu et al. found that a combination of Xanthan Gum (XG), a natural biopolymer, and silica nanoparticles produces an effective dispersant. Nanoparticles disperse oil through a Pickering emulsion. The XG is added because it promotes the nanoparticles to be further adsorbed at the oil-water interface, significantly increasing the stability of dispersed oil droplets [42]. Another similar synergy that has been investigated is silica nanoparticles with rhamnolipids, a bacterial surfactant [33]. These synergies with nanoparticles enhance dispersion effectiveness and also reduce the amount of nanoparticles needed to disperse oil. This is hypothesized to allow a more effective and more economically favorable oil dispersant than nanoparticles alone, while still being biocompatible [32, 42] [33], [42].

Nanoparticle dispersants are also being researched through a design that grafts a nanoparticle core with amphiphillic polymers in order to form unicellular “nanoparticle micelles” [43]. These nanodispersants have the ability to sequester hydrocarbons and stabilize the dispersed oil droplets in water. Conventional dispersants consist of a hydrophobic tail (which remains in the oil) and a hydrophilic head (which partitions into the water) to disperse the oil droplet. The head and tail formation around the oil droplet causes the surfactant to be free and unaggregated. These characteristics allow for the surfactants to be susceptible to removal from dispersed droplets due to dilution. Once the surfactants are removed, the oil droplets destabilize, coalesce and aggregate, and eventually the drops become large enough to rise out of the water column. Dilution is inevitable in the ocean and the instability of surfactants raises concerns on the long-term effectiveness of traditional dispersants when applied in the real world. Experiments found that these micelles are not as susceptible to the disaggregation from dilution that traditional dispersants are prone to. This is due to the micelles being unicellular and amphiphillic, meaning they are only made up of a single molecule (unlike a head and tail) that is able to encapsulate oil and stabilize droplets in water. The micelles are also structured by covalent bonds, which are only degraded chemically. These micelles are being further studied to determine how the polymers (highly branched amphiphillic “star” polymers) and nanoparticles (amphiphilic grafted nanoparticles) used to synthesize the nanodispersants can be further modified to increase dispersant performance, biocompatibility, and also be an economically viable option for oil spill remediation [43, 44] [43] [44].

Polyamidoamine (PAMAM) dendrimers have been researched for both dispersion effectiveness and toxicity to aquatic organisms. Low concentrations (1 μM) of the cationic generation-4 PAMAM dendrimers were non-toxic to the soil amoeba *Dictyostelium discoideum*. However, when these dendrimers were tested at high concentrations (10-50 μM) they did exhibit cytotoxicity. This toxicity resulted from the positive charge of the dendrimers. In the case of the amoeba, cell membranes became depolarized due to the positive charge and resulted in the cells uptaking the highly cationic PAMAM dendrimers. It is unknown whether cellular uptake would occur in other organisms, such as bacteria, but adsorption onto and disruption of the cell membrane is quite possible, due to the positive charge of the polymers. It is believed that a change in charge of the dendrimer to neutral or negative will significantly decrease the toxicity of the dendrimers [45]. Many studies have documented that as a PAMAM dendrimers' surface charge is decreased to a neutral charge, the toxicity of the dendrimers is also decreased [46-48]. This same idea can be applied to other positively charged polymers that exhibit oil dispersing properties [45].

Polymers have been proposed as oil dispersants because their individual molecular architecture can be modified to exhibit properties that can be utilized for oil dispersion [49]. Hyperbranched polymers (HBP's) are polymers with a high density of functional groups present in a 3-D dendritic structure. HBP's have characteristics that make them more capable oil dispersion than other polymers. Unlike linear polymers, HBP's have a 3-dimensional structure that prevents the polymer chains from becoming entangled. Also in contrast to linear polymers, they can have a good solubility and low viscosity. They also have many terminal functional group possibilities that can easily be chemically modified [50]. In addition to end group modification, backbone and hybrid modification can also be achieved. These many possibilities, allow for HBP's to be tailored for specific purposes. A lot of on going research is devoted to making the synthesis of HBP's cost effective and to being able to further understand how the structures of HBP's can be modified in order to perform a specific function [49].

HBP's also have the potential to be both biocompatible and biodegradable. In fact, some HBP's have been investigated for various therapeutic applications due to their low toxicity, non-immunogenicity, and their ability to be easily degraded and metabolized. There are currently seven major types of HBP's that have been studied for use in various therapeutic applications: polyethers, polyesters, polyphosphates, polysaccharides, polypetides, polyamines, and others. Hyperbranched polyethylenimine (Hy-PEI) polymers, visualized in **Figure 2-3**, are a type of hyperbranched dendritic polymer that have exhibited properties that could be utilized for oil dispersion with a potentially lower toxicity than Corexit [51]. Hy-PEI polymers can be produced at a variety of molecular weights. **Figure 2-3** represents all sizes of the polymers, with dotted lines indicating the addition of further branching for each respective size.

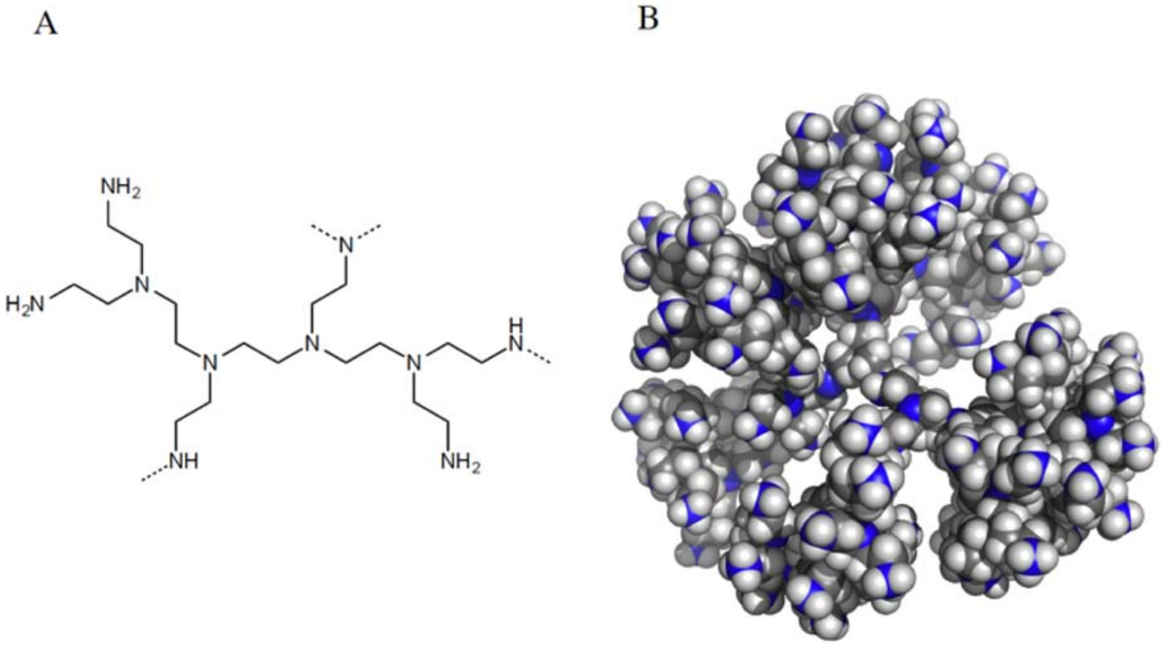

Figure 2-3. (A) Hy-PEI polymer chemical structure representative for every size Hy-PEI available. Only a portion is shown; dotted lines indicate the connections to further branches. (B) A model of an example Hy-PEI polymer, showing its globular structure. Blue indicates nitrogen, black is carbon, and white is hydrogen.

Hy-PEI polymers were found to be able to disperse based on a hydrocarbon bridge model, shown in **Figure 2-4**. It is believed that the Hy-PEI polymers are linked to the crude oil drops through the hydrocarbons present in the oil. The hydrocarbons penetrate the polymer's surface and then are able to adsorb inside the polymer. In addition, these hydrocarbon bridges may also allow the polymer itself to adsorb onto the oil droplet surface. This adsorption reduces the oil/water interfacial tension and is able to further stabilize the oil droplet emulsion [51, 52], [51] [52] similar to how surfactants work in chemical dispersants.

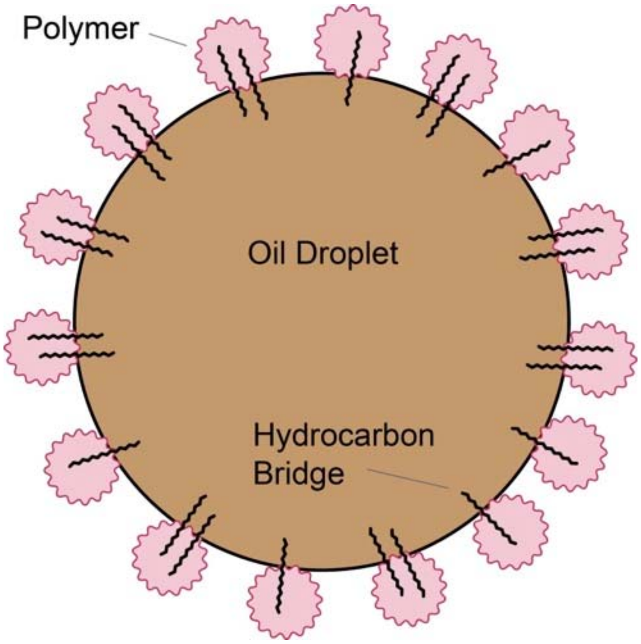

Figure 2-4. Schematic showing the hydrocarbon bridge model of how dendritic polymers may interact with crude oil [51].

Unfortunately, not much specific toxicity data is available on Hy-PEI or many other polymers and it is hard to generalize what makes a polymer toxic versus non-toxic. In addition, all polymer data is developed and distributed by the manufacturer. These data often do not supply much toxicity information, if any [53]. In order to determine the toxicity, separate studies must be completed. It has been determined that charge and molecular weight are related to biological activity toxicity and that it is the cellular accumulation of the polymer that cause this toxicity [36]. The Hy-PEI polymers have undergone toxicity testing with the freshwater organism, *Daphnia magna*. These polymers still exhibited acute toxicity to *D. magna*, but the Hy-PEI with a molecular weight of 10 kDa had a lower toxicity than Corexit [54].

The research presented in this thesis continues the study of polymers as oil dispersants. The dispersion effectiveness of both Hy-PEI polymers and Corexit are tested to see how each dispersant is affected by various parameters: dispersant concentration, salinity, and pH. In addition, new polymers with polyethylene glycol core and polyester hyperbranching (Hy-PE-PEG polymers) were also subjected to dispersion effectiveness tests to determine their capability for oil dispersion in comparison to both Corexit and the previously examined Hy-PEI polymers.

2.3 Measuring Dispersion Effectiveness

Various protocols for testing the effectiveness of dispersants have been developed and studied. In September of 1994 the US Environmental Protection Agency (EPA) adopted the swirling flask test (SFT) as the official, standardized protocol. However, after reexamination it was found that the SFT can result in large error when tested by different laboratories. This led to the development of the baffled flask test (BFT) as a new effectiveness protocol. After much testing of the BFT it was found that the BFT showed higher reproducibility and repeatability than the SFT. Due to this the EPA replaced the SFT with the BFT as the new standardized protocol to test dispersant effectiveness in late 2011.

In a desire to use a technique that required less volumes of expensive materials, Tu [51] attempted to design a new protocol to this affect. After many variations Tu developed an adequate protocol that demonstrated high repeatability. Though Tu’s protocol still required the same material volumes, it did not involve the purchase of specialized glassware that is required to run the BFT.

RESEARCH OBJECTIVES

This project builds on work previously performed in our laboratory where HBPs were initially investigated as novel oil dispersants and methods were developed for their testing [51]. The goal was to determine the dispersion effectiveness of HBP as oil dispersants and study the various parameters that could affect dispersant potential. Specifically, the objectives of this new research were:

(1) ***Determine which Hy-PEI polymers have the highest effectiveness in a single water matrix.*** Repeated the effectiveness tests done previously with Corexit and the various sizes of Hy-PEI polymers in an ionic strength and pH condition that mimicked seawater. This was meant to confirm the previous results and show the repeatability of the protocol.

(2) ***Examine how varying dispersant concentration influences dispersant effectiveness.*** The DOR of Corexit and the Hy-PEI polymers were varied in order to observe changes in effectiveness. This helped elucidate the mechanisms of dispersion.

(3) ***Examine how varying water characteristics influences dispersant effectiveness.*** Varied the ionic concentration (salinity) and the pH of the artificial seawater to determine if these properties influenced how well Corexit and the polymers could disperse oil. This also helped elucidate the mechanisms of dispersion, especially with regard to electrostatic interactions affected by ionic strength.

(4) ***Examine any synergies between dispersant concentration and water characteristics on dispersant effectiveness.*** Tested the same range of DOR's in

the various salinity concentrations in order to fully explore the effects of water matrix on dispersion.

(5) ***Compare the test protocol developed in our lab to the EPA standardized (BFT).*** Repeated dispersant effectiveness testing using the BFT protocol. This determined whether our dispersion effectiveness test has comparable results to the BFT. The BFT has been chosen by the EPA to be the standardized test for dispersant effectiveness.

(6) ***Test the effectiveness of a novel set of HBP's with a polyester structure.*** Various polyester-based HBP's were tested for oil dispersion capability because polyester linkages have shown a high potential for biodegradability [55].. The polymers used here also had neutrally charged terminal end groups, which should be less toxic (more biocompatible) than the positively charged end groups of the Hy-PEIs tested previously.

3 MATERIALS AND METHODS

3.1 Artificial Waters

All experiments were done in both artificial freshwater and artificial seawater. Artificial freshwater was prepared using distilled deionized (DDI) water produced from Type I Millipore® Milli–Q, $NaHCO_3$, $CaSO_4 \cdot 2H_2O$, $MgSO_4 \cdot 4H_2O$. These were added to achieve a calcium to magnesium ratio of 2:1, 10-120 ppm as $CaCO_3$ alkalinity, and160-180 ppm as $CaCO_3$ hardness. HCl was added to adjust its pH to 7.8-7.9. This water composition was selected based on the water chemistry required for the *D.magna* toxicity tests [56]. *D. magna* water was chosen because toxicity tests were performed with Corexit and the Hy-PEI polymers at Southern Alabama in conjunction with this project.

Artificial seawater was prepared using Type I Millipore® Milli–Q water, 35 g/L NaCl, and 0.2 g/L $NaHCO_3$. NaOH and HCl were used to adjust its pH to 8. This corresponds to the average ocean salinity of 35 parts per thousand (ppt) and average ocean pH of 8 [57]. Any dilutions of the artificial seawater were made with the DDI water.

Any pH variations of the waters were achieved via HCl and NaOH.

3.2 Crude Oil

The type of oil used is referred to as Louisiana light sweet crude oil, it was obtained via a crude oil processing facility. The oil was stored at room temperature (22 °C) under a fume hood before all experiments. The mass of 100 μL of oil was consistently found to weigh 84 mg, giving the oil a density of 0.84 g/L.

Light sweet crude oil was chosen because it is the most preferred form of petroleum to refine into gasoline, and the category of oil spilled in the Deepwater Horizon spill. Light sweet oil is categorized as such due to its low sulfur content (<1%), this is the same trait that makes is desirable for gasoline production. [8].

It is important to note that a needle syringe was used to dispense all oil for experimentation. The crude oil was found to cause problems with costly pipettors and could result in the pipettor becoming useless. To prevent this damage, the use of pipettors for the crude oil was avoided as much as possible.

3.3 Oil Dispersants

3.3.1 Corexit

Corexit 9500, furthermore referred to as just Corexit, was obtained via NALCO, Inc. NALCO, Inc. is the manufacturer that produces and sells Corexit as a chemical oil dispersant. It was assumed to be suspended in a 30% aqueous solution because the actual water content was unknown and this was a more conservative estimate. To make working solutions that would add a specific amount of Corexit for a desired DOR for experimentation, the Corexit was added to DDI water in calculated ratios.

Corexit was tested because it was the dispersant used abundantly in the Deepwater Horizon spill. In addition, Corexit is also the most common dispersant used worldwide. Therefore, it makes a good baseline comparison to how well new dispersants tested should work in order to perform as effectively as the current dispersants.

3.3.2 Hy-PEI Polymers

Hy-PEI polymers were purchased from Polyscience and delivered dissolved in an aqueous solution in various percentages. **Figure 2-3** shows the structure of these polymers. The polymers are furthermore identified by their specific molecular weights in kilo Daltons. To make working solutions that would add a specific amount of the Hy-PEI polymers for a desired DOR during experimentation, the stock of Hy-PEI was added to DI water and mixed thoroughly.

3.3.3 Hy-PE-PEG Polymers

Various HBP's with a polyethylene glycol core, polyester links (Hy-PE-PEG polymers), and –OH terminal end groups (neutrally charged polymers) were ordered from the Polymer Factory, shown in **Table 3-1**. These Hy-PE-PEG polymers have various core sizes and pseudo generations. Unlike dendrimer generations, the branching of HBP's are referred to as pseudo generations. Whereas the synthesis of dendrimers results in a very well defined structure with known amounts of branching points (generations), HBP's are synthesized with a collection of monomers that are mixed and allowed to react. There is no specific chemistry with HBP synthesis and the exact number of generations is unknown. **Figure 3-1** and **Figure 3-2** show the structures of the two Hy-PE-PEG polymer pseudo. The individual Hy-PE-PEG polymers are furthermore identified by their generation (G4 or G6) and their core size (6k, 10k, or 20k).

The Hy-PE-PEG polymers were delivered in plastic bottles in powdered from. To make working solutions that would add a specific amount of Hy-PE-PEG for a desired DOR for experimentation, the powdered form of Hy-PE-PEG was added to DI water and mixed thoroughly. Most Hy-PE-PEG's dissolved readily into the DI water for the working solution. However, G6-6 and G6-10 required addition mixing with small stir bars/ stir plates for additional time for all the polymer to be dissolved into the working solution.

Table 3-1. Characteristics of the Hy-PE-PEG's purchased from the Polymer Factory [58].

Core Size	Psuedo-Generation	Theoretical Molecular	Solubility (g/mL)
6k	4	9492	Soluble: ~0.1-0.001
	6	18875	Barely Soluble: ~0.001
10k	4	13501	Soluble: ~0.1-0.001
	6	24598	Soluble: ~0.1-0.001
20k	4	23545	Very Soluble: ~0.1
	6	39774	Very Soluble: ~0.1

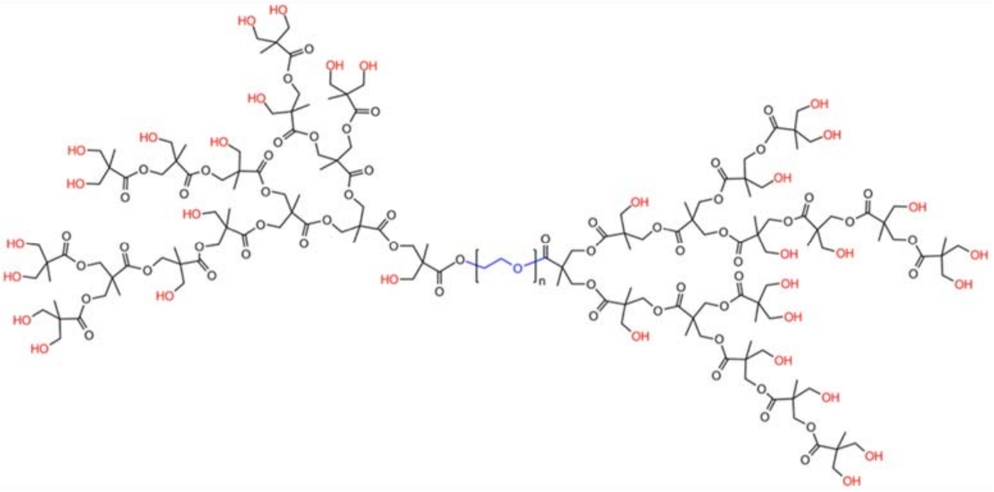

Figure 3-1. Structure of generation 4 hyperbranched Hy-PE-PEG, where "n" denotes the number of repeating units in the core, which were 6,000, 10,000, and 20,000 in this work.

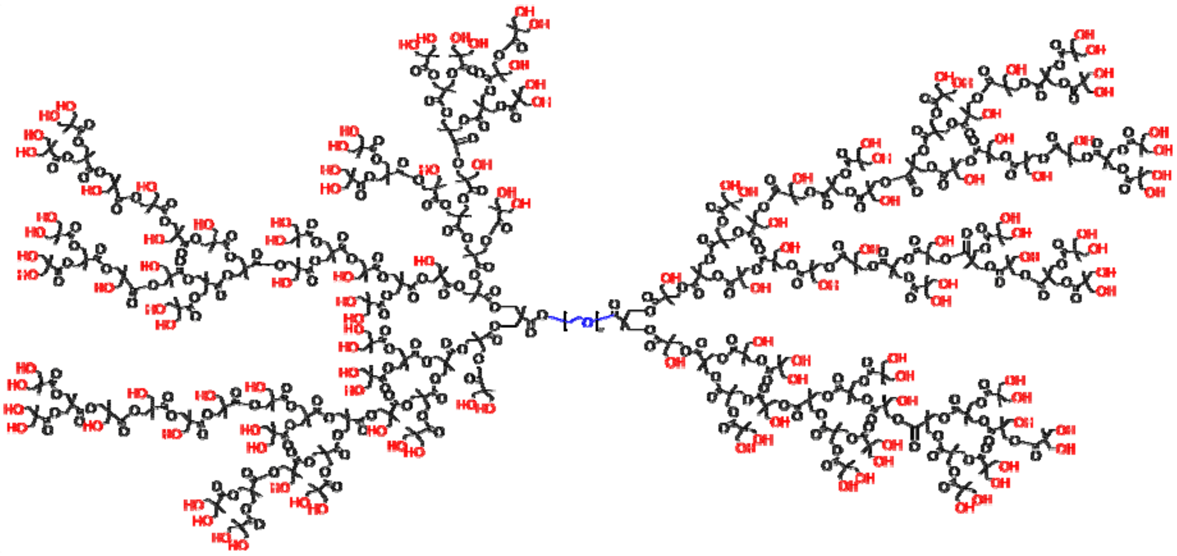

Figure 3-2. Structure of generation 6 hyperbranched Hy-PE-PEG, where "n" denotes the number of repeating units in the core, which were 6,000, 10,000, and 20,000 in this work.

Though it is believed that the Hy-PEI polymers have a reduced toxicity over Corexit they still have some toxicity concerns [54]. Cationic polymers have been shown to be acutely toxic to some aquatic species, like fathead minnows and daphnids [53]. In addition, the toxicity of positively charged PAMAM dendrimers was found to be directly related to this surface charge. A high density of positive charges can cause a lot of harm on the cellular level because the charges can stick to almost any surface. Since the toxicity mechanisms will be the same for Hy-PEI polymers, in order to lower their toxicity, the terminal charge needs to be changed to negative or neutral charged end groups [59]. However, one concern with this is that crude oil is negatively charged and thus having neutral or negatively charged dispersant will lower its dispersion effectiveness.

In addition to the reduced toxicity of the end charge, polyesters were chosen because this branching has been shown to have a high potential for biodegradability. Though Hy-PEI are considered as biocompatible polymers for therapeutic use, polyester linkages are more susceptible to chemical reactions and are also expected to have biodegradable capabilities [55].

3.4 Dispersion Effectiveness Test

Following the procedure developed by Tu [51], 120-mL of water was added to 125-mL screw top bottle. 100 µL of oil was added via a needle syringe to the surface of the water, and lastly a pipette was used to apply different amounts of polymer (to get desired DORs) to the oil slick. The bottles were then shaken on an orbital shaker at 120 rpm for 30 min and poured into separatory funnels, the solution in the funnels was left to settle for 15 min. After settling, ~29 mL of the mixture was drawn into a centrifuge tube, the extraction was repeated three additional times in order to draw out all of the solution, without dispensing the “scum layer” (**Figure 3-3**). In order to insure the correct volume of mixture was dispensed, the centrifuge tubes were placed on a scale while the extraction occurred and filled until the mass extracted was approximately 29 g. After extraction into the centrifuge tubes, 10-mL of dichloromethane (DCM) was then added to the tubes, which were then shaken and allowed to settle. 200 µL of the DCM layer was put into three different microplate wells with a repeating pipettor for each centrifuge tube, The microplate was then put into the Beckman Coulter DTX880 Multimode Detector to measure the absorbance of each well at 340 nm. Readings were taken both days of and after a night of settling to ensure that all the oil in the mixture had been extracted into the DCM layer.

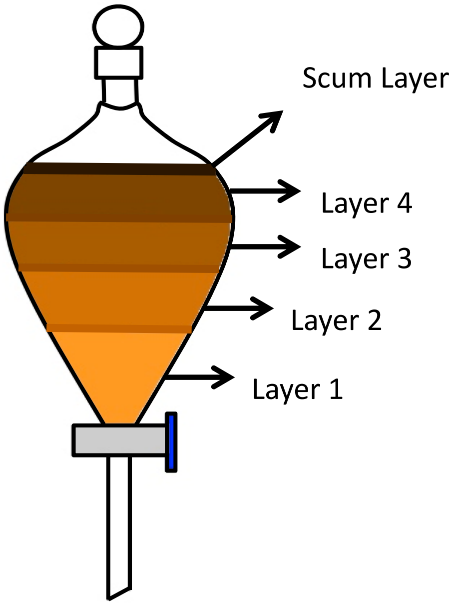

Figure 3-3. An illustration of the four layers and the “scum” layer after the mixture settles in a separatory funnel.

3.4.1 Determining Fraction of Oil Dispersed from Absorbance

In order to develop a relationship between absorbance at 340 nm and the amount of oil present a standard curve was developed. Known volumes of oil were completely dissolved into 10 mL of DCM and then 200 µL of the DCM/oil mixture was put into three different microplate wells with a repeating pipettor and absorbance was measured with the microplate reader at 340 nm (to mimic the 10 mL DCM volume used to extract oil and microplate readings that are performed in effectiveness tests).

From the standard curve shown in **Figure 3-4**, absorbance values gathered from the dispersion effectiveness can be related to a volume of oil in 10 mL of DCM. This value is then used to calculate the fraction of the 100 µL of oil that is dispersed in each sample.

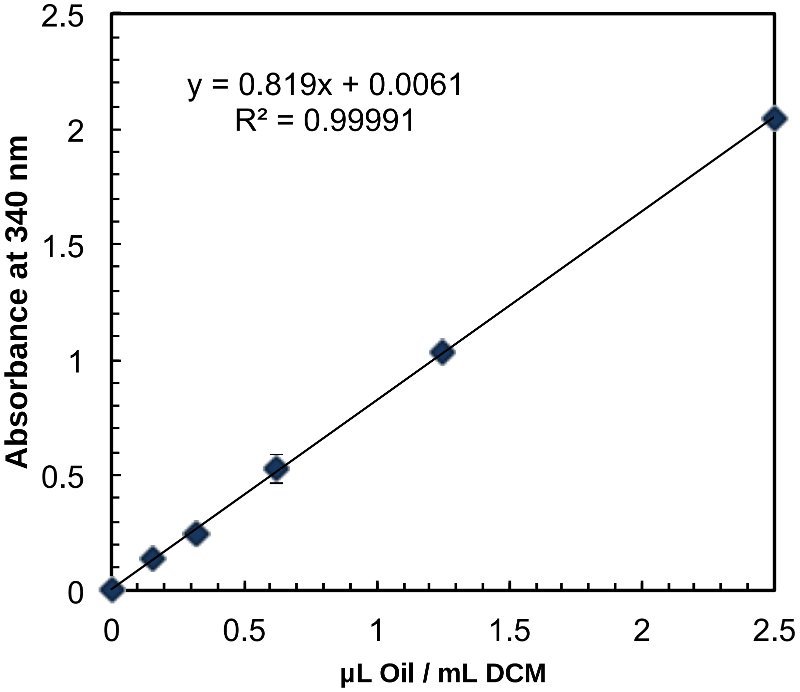

Figure 3-4. Relationship between known volumes (µL) of oil in 10 mL of DCM and its corresponding absorbance at 340 nm. The line represents a linear trend line fit to the data and is used as the standard curve to relate absorbance to volume of oil present.

3.4.2 Drop Coalescence

If the oil drops are not adequately stabilized, the oil will begin to coalesce and rise to the top. Drawing out the mixture in four different increments allows the tracking of how much oil is rising to the top, and how much is truly dispersed in the solution. A dispersant that is the most effective will have stabilized drops and consequently have oil evenly dispersed through the four levels. **Figure 3-3** illustrates the four 30-mL layers that are drawn out of the separatory as well as the scum layer that is left in the separatory funnel because this oil is not considered to be dispersed.

3.4.3 DCM Extraction

DCM was used to extract the oil. It is an organic solvent that is miscible with oil, but not water, making it ideal to collect all oil that was dispersed in the artificial waters. DCM was also used in order to ensure all glassware was properly cleaned between experiments and no oil residue was left that would contaminate future experimentation. Great care was taken when using DCM because it poses health hazards through inhalation and adsorption. Also, the use of DCM in experiments meant that all experimental waste was hazardous and had to be disposed of properly.

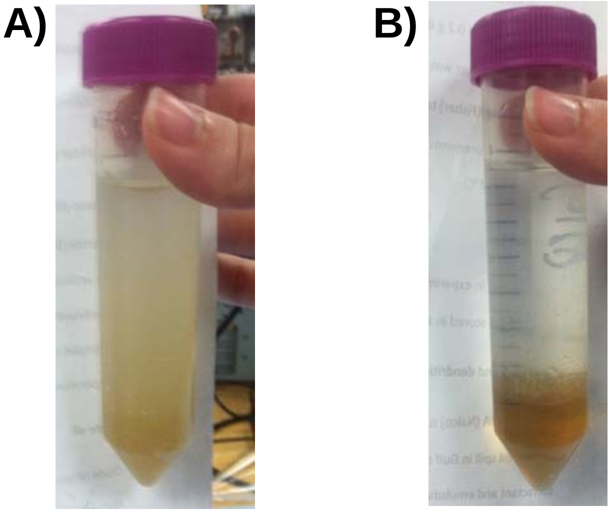

Figure 3-5. Photos showing a dispersed mixture in centrifuge tubes (A) before and (B) after DCM extraction.

3.4.4 Absorbance Readings

Absorbance readings were performed via the Beckman Coulter DTX880 Multimode Detector and LabView software with a 96 well plate. A LabView program was designed to take the visible light absorbance of each well at 340 nm. First, the machine was zeroed with the plate and its glass cover slip. Then the DCM/oil layer of each centrifuge tube, shown in **Figure 3-5**, was drawn up into an Eppendorf repeating, and 200 µL was dispensed three times into different wells. Each different scenario of the effectiveness test took up one row of the plate, which can be seen in **Figure 3-6**. Once the wells were filled, the plate and its cover slip were inserted and the program read each well's absorbance at 340 nm. This absorbance data was then converted to fraction of oil dispersed (dispersion effectiveness).

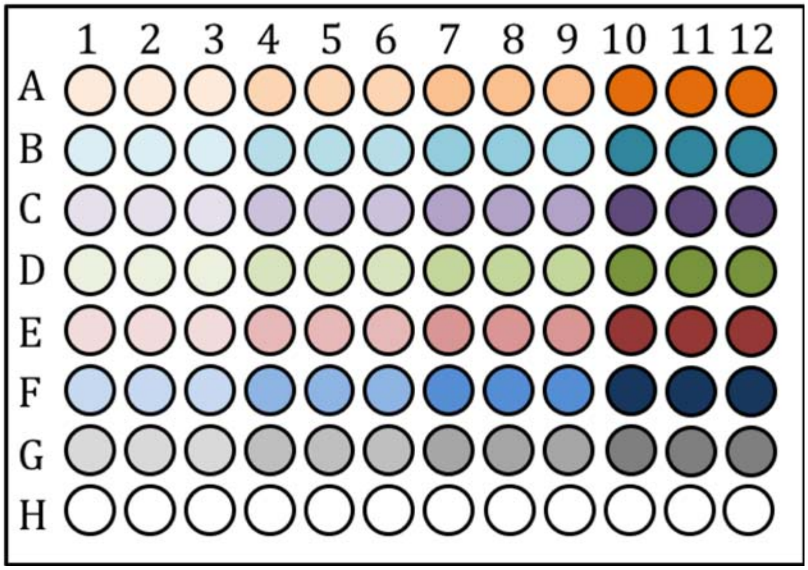

Figure 3-6. A schematic of the procedure to fill the well plate. Different colors represent different dispersants or scenarios run. The increase in color darkness signifies the trend that more oil was usually present in the higher volume extractions from the separatory funnels.

In order to ensure that the evaporation of DCM did not occur to an extent that would affect the absorbency readings, the plate was continuously covered with its glass cover plate while filling the wells. In addition, a maximum of two rows (two dispersants) were filled for a single run to help further reduce DCM evaporation.

3.5 Varying Parameters

The DOR of each dispersant as well as the salt concentration and pH of the water were varied in order to determine what effects these had on dispersion effectiveness. DOR was varied from zero to 1:1 by simply adding more dispersant to the oil slick in the shaker bottles before shaking and settling. Various salt concentrations were obtained by diluting the artificial seawater with DI water. Initial pH of the artificial seawater was varied before oil and polymer addition. In addition, in a few runs pH was measured before oil/polymer addition and after separatory funnel settling to see if the dispersants caused in pH changes.

3.6 Oil Droplet Size

To attempt to further visualize the effectiveness of the Hy-PEI polymers, pictures of water, oil, and dispersant mixtures were taken in order to see how the Hy-PEI polymers changed the size of the oil droplets suspended in water. Light microscope images were taken of the mixture of the water, oil, and polymers. 120-mL artificial seawater, polymer at a 1:50 (0.02) DOR, and oil at a 1:1200 (0.00083) OWR were mixed in a 125-mL bottle for 30 min at 200 rpm; same as the protocol for the dispersion effectiveness tests. The mixture was then pipetted onto a microscopic slide. A picture of this droplet was taken via a camera connected to a light microscope at 40x magnification. The pictures were also taken in phase contrast in order to enhance the drop images.

3.7 Interfacial Tension Measurements

All interfacial tension (IFT) measurements were obtained via the Krüs Easydrop machine with Drop Shape Analysis (DSA) software. The instrument consists of a camera, 10x10x10 cm glass cuvette, backlight, and a syringe/needle system that delivers oil droplets into the water/dispersant solutions, the set up is shown in Figures 3-7 and 3-8.

The camera takes live video of the oil droplets **(Figure 3-7**), and the drop images are analyzed via the DSA software which uses the Young-Laplace calculation to calculate the drop's IFT measurements versus time (**Figure 3-8**).

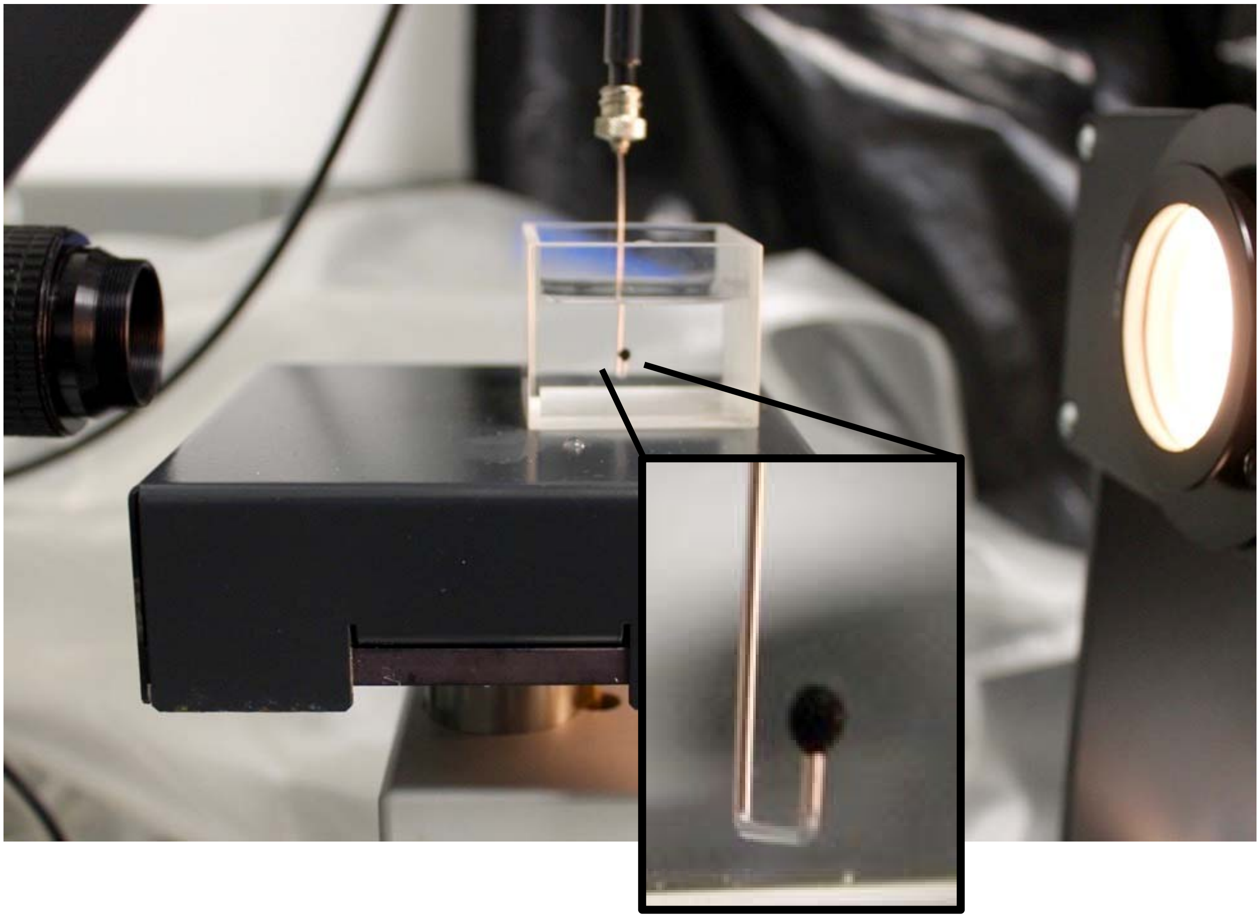

Figure 3-7. A photo showing the Krüs Easydrop setup with the camera, cuvette, syringe/J-needle dispensing system, and light. The inset shows an up-close view of the J-needle and oil droplet.

Figure 3-8. A screenshot of the live video of the oil droplet that is analyzed by DSA to determine the IFT between the oil droplet and water.

The DSA method chosen was the floating pendant drop method measuring from bottom to top. Meaning the oil droplet rose up from a J-shaped needle, with a 0.632-mm diameter, and its IFT was measured as a 3-μL oil droplet was dispensed and became attached to the needle until the oil drop was released. The oil drop changed shape over time due to decreasing IFT and eventually got released when the IFT became too low. The water solution in the cuvette was either artificial seawater or freshwater mixed with the each of various dispersants at the concentrations of 12.5, 25, 50, or 100 ppm. All solutions were prepared immediately prior to experimentation and measurement. Each of these experiments was done in triplicates, two done on different days.

Before running each experiment it was essential to make sure the needle was free of all oil contamination. If any oil was present on the needle surface, oil released would not form a bubble suspended in the liquid, but instead would become attached to the oil contamination. In order to ensure the needle cleanliness, a Q-tip with DCM was used to wipe down the needle in between each experiment. Then a dry paper towel was used to remove any DCM that may be left on the needle. Also, to ensure that the oil inside the needle was not contaminated with DCM, three oil droplets were released before IFT measurements were taken.

3.8 Baffled Flask Tests

The Baffled Flask Test (BFT) is currently considered to be the most accurate laboratory dispersant testing protocol by the EPA. The EPA adopted the BFT as the official EPA dispersion effectiveness test in 2011. It replaced the Swirling Flask Test (SFT) that had been in use since 1994 because the BFT was shown to produce better results with a much higher reproducibility [60]. There are several key differences between Tu's protocol and the BFT. The first is the glassware used for shaking and settling. Whereas Tu's uses 150-mL screw top bottles for shaking and separatory funnels for settling, the BFT uses 150-mL baffled flaks. These flasks used are modified with a stopcock as shown in **Figure 3-9** [61, 62] [61] [62]. Flasks with baffles were chosen because the baffles add the potential for greater mixing energy [60]. There are also differences in the determination of calibration curves, the extraction method, absorbance measurements, and calculation of dispersion effectiveness.

Figure 3-9. A baffled flask modified with a stopcock, used for the BFT.

3.8.1 Calibration Curve Preparation

Unlike Tu's protocol, the BFT protocol calls for developing an individual standard curve for each different dispersant being tested. First a stock standard solution of the oil and individual dispersant must be prepared.

A clean 20-mL glass-vial with a screw cap was weighed and the weight was recorded. 2-mL of the LSC oil was added to the vial, and the oil + vial + cap was weighed and recorded. Then 80-μL of dispersant stock solution was added in order to achieve a DOR of 1:25 (0.04), and the dispersant + oil + vial + cap was weighed and recorded. Lastly 18-mL of DCM was added to the vial and the DCM + dispersant + oil + vial + cap was weighed and recorded. The density of this stock standard solution was calculated by the recorded weights and added volumes. Once the stock standard solution was made, the standard solutions for the corresponding calibration curves were created.

Each calibration curve consists of six points of different oil/dispersant concentrations (six different volumes of the stock standard solution). For each standard solutions, the BFT SOP has different specific volumes based on the type of oil being tested. The specific volumes for Louisiana Crude Oil are 20-µL, 50-µL, 100-µL, 150-µL, 200-µL, and 300-µL. To create the six-point calibration curve, 30-mL of artificial seawater was poured into a 125-mL glass separatory funnel, then the specific volume of the stock standard was added to the funnel.

5-mL of DCM was then added directly to the separatory funnel to extract the standard. The funnel was mixed by hand for 15 seconds and then allowed to settle for 2 min. After settling, 3-mL of the DCM layer was drained into a 25-mL graduated cylinder. This extraction process was done two more times with 5-mL of DCM added to the separatory funnel, shaken, settled, and then extracted to the DCM/water interface into the 25-mL graduated cylinder. It is important to drain all of the DCM without allowing any water to drain from the separatory funnel.
After the three DCM extractions, the final extract volume was adjusted to 20-mL with addition DCM. This extract was then poured into a glass screw-top vial, sealed tightly, inverted 10 times, and stored in the refrigerator (4 ±°1 C) until all samples were prepared and ready for analysis. With the DCM extraction it was important to be careful with the building pressure the DCM exerted on the top of the separatory funnel, pressure should be released frequently and carefully. An example of the vials prepared for each calibration curves is shown in standard curves for each polymer run during experimentation can be found in **Appendix A**.

Figure 3-10. The vials containing the blank followed by the six solutions used for Corexit's calibration curve.

3.8.2 Sample Preparation

Two modified baffled flasks were available for experimentation, so each sample was run in duplicate. 120-mL of artificial seawater was added to the baffle flask. 100-µL of crude oil was added to the water surface with a syringe. Then the dispersant was added to the center of the oil slick to give a 1:25 (0.04) DOR; it is important to make sure the dispersant is added onto the oil slick and not directly into the water. The baffled flasks were then sealed and shaken on an orbital shaker for 10 min at 200 rpm. After shaking, the contents of the flasks were allowed to settle for 10 min.

Once settling was complete, the screw top was opened and 2-mL from each baffle flask was drawn from the stopcock and discarded. Then 30-mL of the sample was drained from the stopcock into a graduated cylinder. This 30-mL was then poured into a 125-mL glass separatory funnel. The same three 5-mL DCM extractions as described for the calibration standards are completed with the sample. Once the sample has been extracted and adjusted to 20-mL with DCM, the vial were carefully sealed and stored in the refrigerator until all samples are ready to by analyzed.

3.8.3 Absorbance Readings

The spectrophotometer was turned on and allowed to warm up for 30 min. All calibration standards and samples were removed from the refrigerator and brought up to room temperature prior to analysis. Before absorbances could be taken, a blank was prepared via the same protocol as the sample preparation.; only artificial seawater was added to the separatory funnel and extracted three times with 5-mL of DCM (no shaking necessary for the blank).

Each calibration standard and sample were measured at three absorbances: 340, 370, and 400 nm. A specific sequence of analysis was followed for each dispersant sample at each wavelength: blank, six calibration standards, two replicates. It was important to have a clear way to record the many absorbance values gathered for proper analysis.

3.8.4 Calculations

Calculations to convert the absorbance values gathered to total dispersion effectiveness were laid out in the EPA's SOP for the BFT. First the concentration of the oil in stock solution must be calculated using **Eq. 1**:

$$\boldsymbol{C} = \frac{\boldsymbol{y-x}}{\left(\boldsymbol{z-x}/\boldsymbol{\rho}\right)} \tag{1}$$

C: concentration of oil (g/L)
x: clean vial with cap
y: clean vial + cap + 2 mL oil + dispersant
z: clean vial + cap + oil + dispersant + 18 mL DCM
ρ: density

The slope of each calibration curve must then be calculated using **Eq. 3** via the area under the curve from each absorbance and the concentration of oil added which can be calculated with **Eq. 2**

$$\boldsymbol{y} = \frac{(\boldsymbol{Abs_{340}+Abs_{370}})*\boldsymbol{30}}{\boldsymbol{2}} + \frac{(\boldsymbol{Abs_{370}+Abs_{400}})*\boldsymbol{30}}{\boldsymbol{2}} \tag{2}$$

$$\boldsymbol{y = mx} \tag{3}$$

y = Area under absorbance curve
x = Concentration of oil added from standards (g/L)
m = slope of calibration curve

Once the slope of the calibration curve has been determined, the dispersant performance (perfect for oil dispersed) of the experimental samples can be calculated via the sample's absorbance area. First by determining the concentration of oil in the sample with **Eq. 4**:

$$\boldsymbol{C = \frac{y}{m}} \tag{4}$$

Then the mass of oil dispersed in the 30-mL of extracted sample can be calculated with **Eq. 5**:

$$\boldsymbol{M = C * V_{DCM}} \tag{5}$$

M = mass of oil (g)
V_{DCM} = final volume of DCM added to extract sample = 0.02 L

The mass of oil dispersed in the sample can then be determined via **Eq. 6**:

$$\boldsymbol{M_D = M * \frac{V_{tw}}{V_{ew}}} \tag{6}$$

M_D = mass of oil dispersed (g)
V_{tw} = water volume added to separatory funnel (120 mL)
V_{ew} = volume of water extracted from separatory funnel (30 mL)

These values can then be used to determine the dispersant performance in fraction of total oil dispersed with **Eq. 7**:

$$\boldsymbol{F_D = \frac{M_D}{\rho_{oil} * V_{oil}}} \tag{7}$$

F_D = fraction of total oil dispersed

An example of the values from these calculations is shown in **Table 3-2**.

Table 3-2. Calculation for determining the fraction of oil dispersed by the Hy-PEI 70 kDa polymer after performing the BFT protocol.

Area	Calibration Slope	Oil Concentration (g/L)	Mass Oil Dispersed (mg)	Total Oil Dispersed (mg)	Fraction Oil Dispersed
22.995	38.151	0.603	12.055	48.219	0.578
23.925		0.627	12.542	50.169	0.601

4 RESULTS AND DISCUSSION

4.1 Hy-PEI Polymer Effectiveness

The first tests repeated Tu's experiments [51]. Tu found that in artificial seawater effectiveness was highest with Hy-PEI 10 kDa followed by Corexit, Hy-PEI 70 kDa, Hy-PEI 750 kDa, Hy-PEI 1.8 kDa and Hy-PEI 1.2 kDa; the latter two had very little dispersion capability. As shown in **Figure 4-1**, the same trend was observed in the new set of data. It appears that the polymers need to be sufficiently large in order to be able to disperse oil; however, if the polymers are too large, they may interact with and fold in on themselves, hindering their dispersion ability. This leads to a destabilization of the oil droplets, drop agglomeration, and decreased dispersion.

Figure 4-2 shows the total dispersion effectiveness data gathered during Tu's experimentation compared to the new data collected in 2016. Though the trends of most efficient polymers are the same, 2016 shows effectiveness data that is lower overall than what was reported by Tu. This could be due to differences in experimenter. In addition, the oil and polymers had undergone additional aging from when they were first acquired for Tu's experimentation. The oil may have lost volatile components and the polymers may have experienced changes from age that could be responsible for the slightly lower effectiveness variations seen in the 2016 data. Additionally, the discard of the scum layer (seen in **Figure 3-3** was not a part of Tu's original protocol, the entirety of the separatory funnel's water column was withdrawn into the centrifuge tubes. This slight change in protocol is another reason for the lower effectiveness measurements seen in 2016.

Another method changed from Tu's procedure was that absorbance measurements of the DCM layer were taken both on the same day as the extraction and after a night of settling in order to insure that all of the oil had truly been captured into the DCM layer. The day-after readings consistently had lower standard deviation than their day-of counterparts; thus, the day-after readings are reported here.

For most dispersant effectiveness graphs, effectiveness was graphed versus the average water column volumes. These volumes refer to the four different layers that were withdrawn from the separatory funnel, as shown in **Figure 3-4**. Layer 1 is 0 to 30 mL, Layer 2 is 30 to 60 mL, Layer 3 is 60 to 90 mL, Layer 4 is 90 to 120 mL; these volumes were averaged to get the values of 15, 45, 75, and 105 used for data presentation in the figures. Dispersion effectiveness was presented this way to show how the oil droplets rose during settling and to be able to further visualize how well the dispersant dispersed the oil throughout the entire water column. Total dispersion effectiveness was calculated by adding the four fractions of oil dispersed in the four layers.

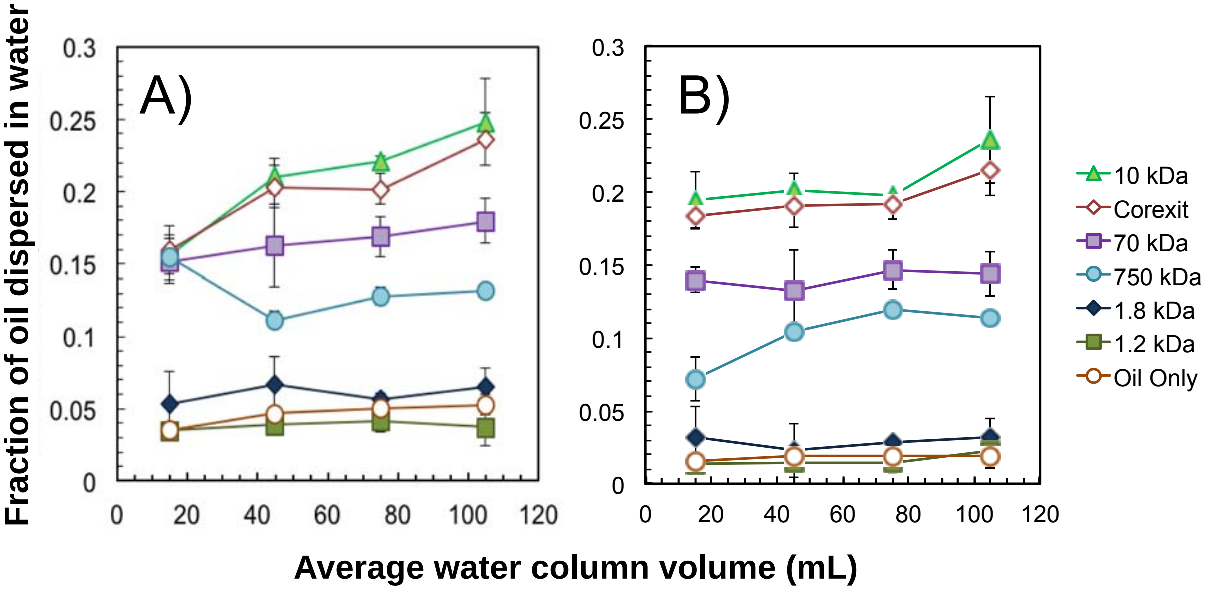

Figure 4-1. Oil dispersion effectiveness data for Hy-PEI polymers compared with Corexit and a crude oil control (no dispersant) in artificial seawater from (A) Tu's data in 2014 and (B) the same protocol run in 2016. Error bars represent the standard deviations from three distinct experiments. Absent error bars are smaller than the symbols.

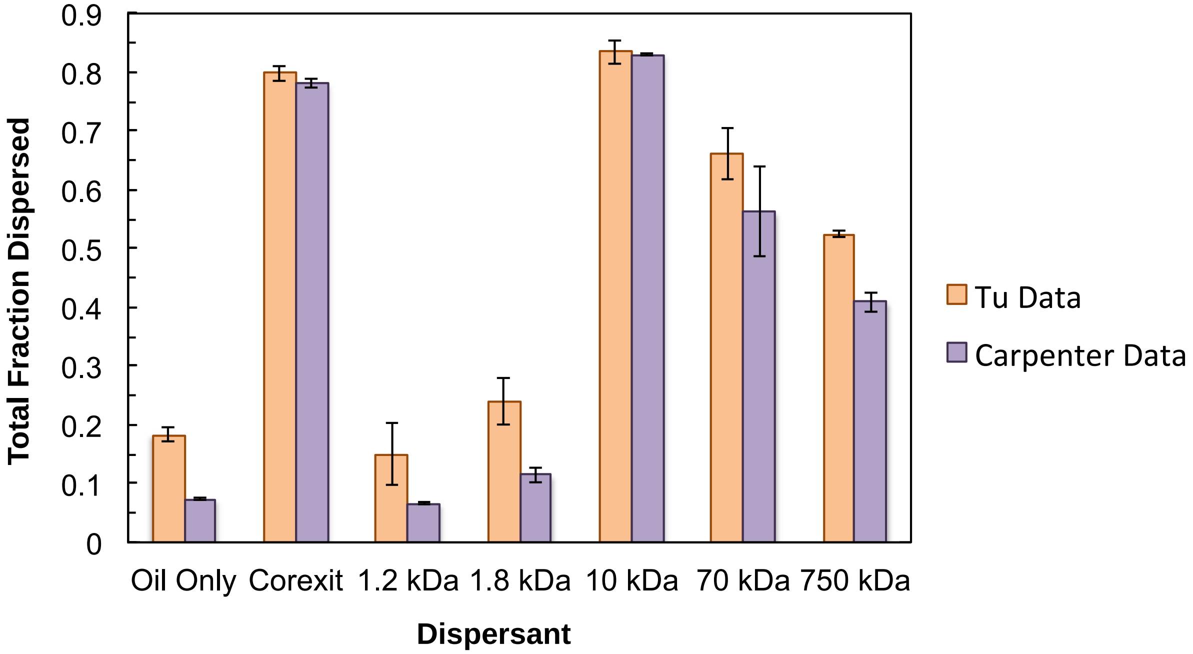

Figure 4-2. Total dispersion effectiveness data comparison between Tu's 2014 data and the same protocol run in 2016. Experiments were done at a 1:50 DOR and 35 ppt salinity.

In order to compare dispersion effectiveness to toxicity studies done with *Daphnia* [54], these effectiveness experiments were repeated in artificial freshwater in which *Daphnia* are grown. The results are shown in **Figure 4-2**. The comparison of total dispersion effectiveness for each polymer in artificial seawater versus artificial freshwater is seen in **Figure 4-3**.

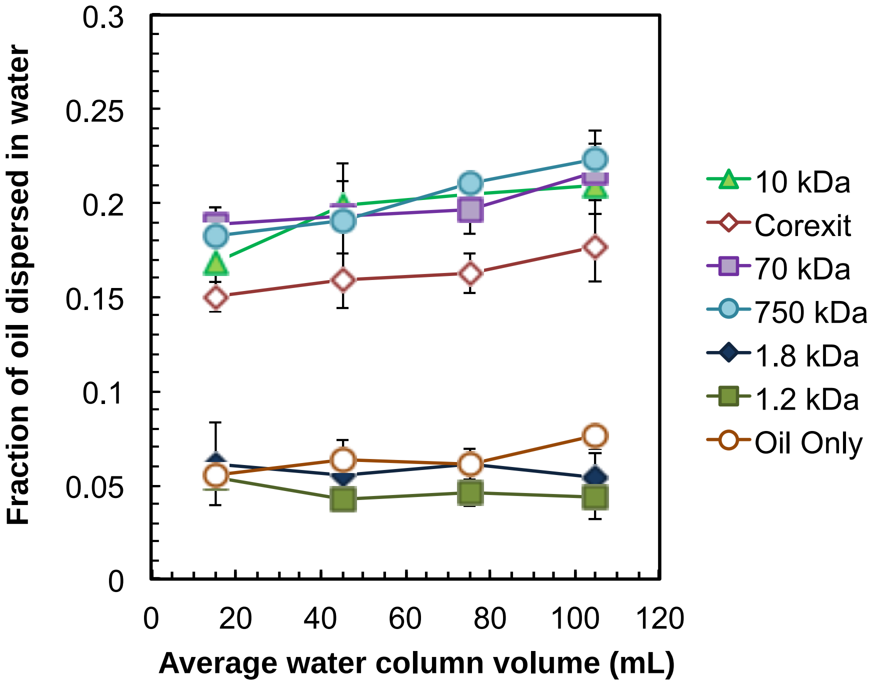

Figure 4-3. Oil dispersion effectiveness data for Hy-PEI polymers compared with Corexit and a crude oil control (no dispersant) in artificial fresh water. Error bars represent the standard deviations from three distinct experiments. Absent error bars are smaller than the symbols.

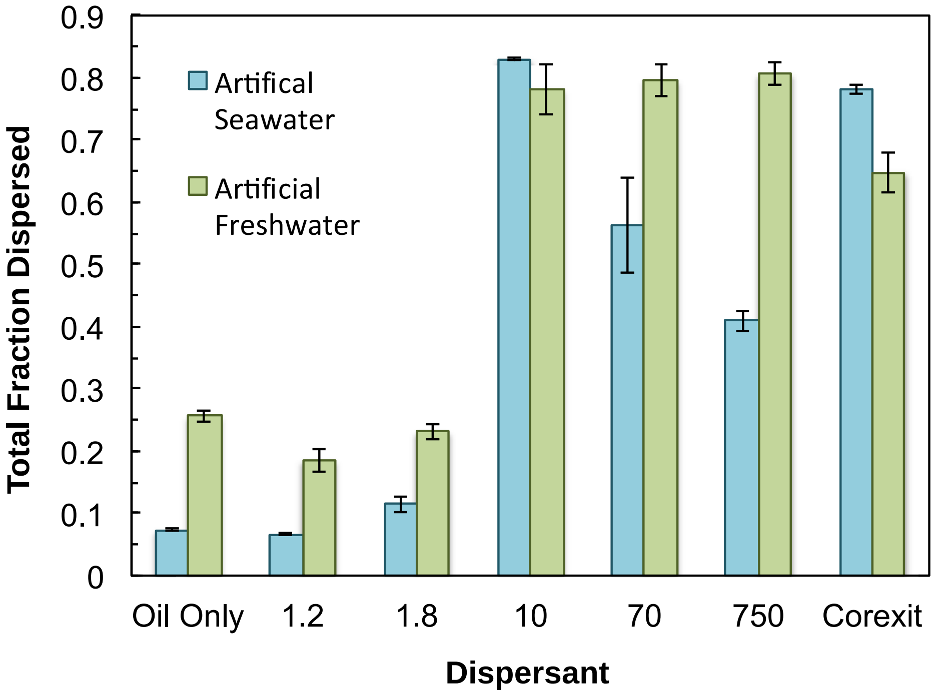

Figure 4-4. Comparison of the total dispersion effectiveness for Hy-PEI polymers and Corexit in both artificial seawater and artificial freshwater. Error bars represent the standard deviations from three distinct experiments.

In artificial freshwater, 1.2 and 1.8 kDa still had no dispersion capabilities. A major difference in trends came with 70 and 750 kDa: whereas in artificial seawater these larger molecular weights had lower dispersion capabilities than Corexit and 10 kDa, in the artificial freshwater they exhibited the same effectiveness as 10 kDa. This could be because the higher molecular weight polymers are less stable in high-ionic-strength saltwater, but more stable in low-ionic-strength freshwater. Greater polymer stability would lead to more stable oil dispersions.

The stability phenomena can be attributed to double layer compression as explained by the theory developed by Derjaguin, Landau, Verwey, and Overbeek (DLVO theory). Having more ions in the water decreases the double-layer thickness for the oil droplets and polymers. As the double layer is compressed the repulsive energy between oil droplets and the polymers is reduced. This reduction in repulsive energy allows for the van der Waals attraction forces between the oil droplets and polymers to dominate. These attractive forces leave the polymers extremely susceptible to aggregation and flocculation, making them inadequate as oil dispersants. Without the polymers acting as a dispersant, oil droplets are very easily attracted together, coalesce, and become large enough to rise out of the water column, no longer being dispersed [63].

Corexit's dispersion results had the opposite trend as Hy-PEI; Corexit was more effective in artificial seawater than freshwater. This is because—along with most other chemical dispersants—Corexit is specifically designed to work at salinities of the open ocean (~35 ppt). In freshwater, the surface tension between water and oil is higher (seen in **Figure 4-10**) and surfactants become more lipophilic. Being more lipophilic, the surfactant is less soluble in water and its HLB is decreased. A lower HLB promotes water in oil emulsions, which is not ideal for oil dispersion in water. All these effects on surfactants from freshwater significantly decrease the stability of oil droplets in the water, which causes the decrease in Corexit's effectiveness [29].

4.2 Varying Parameters

4.2.1 DOR Variation

Increasing the DOR had different effects on each dispersant's effectiveness as shown in **Figure 4-4**.

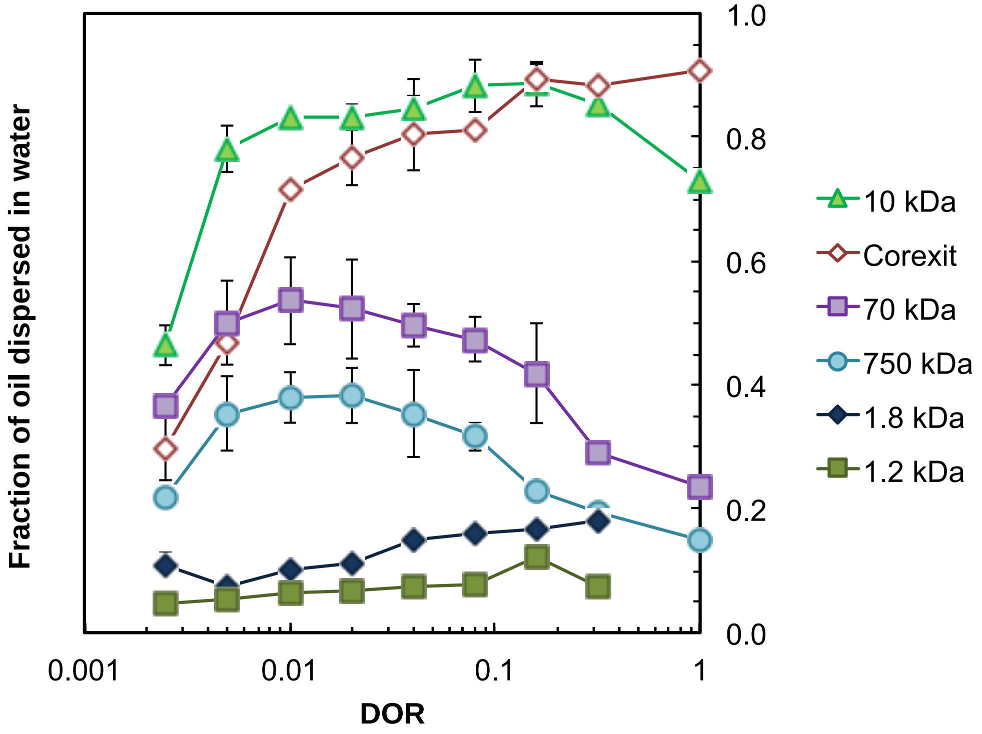

Figure 4-5. Total dispersion effectiveness data for Hy-PEI polymers compared with Corexit based on the DOR of each dispersant added all in 100% artificial seawater. Error bars represent the standard deviations from two distinct experiments. Absent error bars are smaller than the symbols except for 1.2 kDa where only one experiment was run.

Whereas Corexit had a steady increase of dispersion effectiveness with increasing DOR, the Hy-PEI polymers appear to have a DOR with a maximum effectiveness and then a dispersion drop-off as the DOR continues to rise. This process has more of an effect on the higher molecular weight polymers (70 and 750 kDa) and their decrease in dispersion effectiveness begins at a lower DOR. Once again, this could be a result of the polymers beginning to interact with themselves; causing the entanglement of the polymer. This entanglement causes there to be less polymers available to interact with the oil and stabilize the oil droplets. Less stabilization leads to an increased oil drop coalescence, and the eventual rise of the oil droplets out of solution. This behavior is amplified as more of the polymer is added to the solution.

This should not be a large concern for wide spread use because dispersants are most often applied to oil slicks at an average DOR of 1:20 (0.05) [26]. Even if there is not extreme control of DOR, the dosages should not reach extremes where dispersion effectiveness begins to severely diminish. These results were more useful in further determining the properties and mechanisms that the various polymers use to disperse oil.

4.2.2 Salinity Variation

Seawater variation was done by diluting artificial seawater to various fractions with DI water. For each dispersant, a range of DORs was tested in the various fractions of artificial seawater; the higher the fraction of artificial seawater, the more ions were in solution. No dilution of the artificial seawater corresponds to the saltwater concentration of 35 ppt. **Figure 4-5** and **Figure 4-6** show these results.

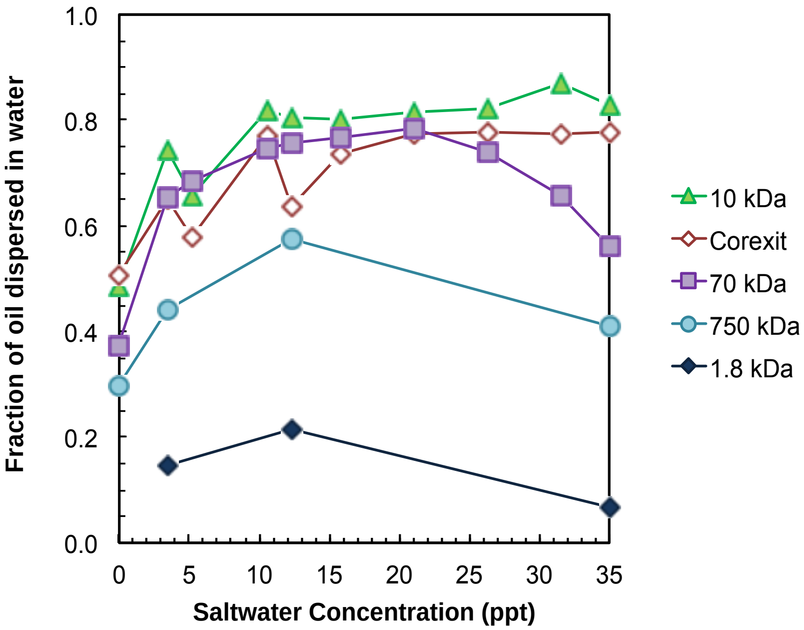

Figure 4-6. Total dispersion effectiveness for Hy-PEI polymers and Corexit versus the fraction of artificial seawater used during effectiveness test at a DOR of 1:50 (0.02).

Figure 4-6 shows that all the Hy-PEI polymers appear to have a maximum salinity, after which a decrease in dispersion effectiveness is seen with increasing salinity. In contrast, Corexit showed a fairly consistent increase in dispersion effectiveness with increasing saltwater concentration. This is consistent with DLVO theory and the properties of Corexit as described previously.

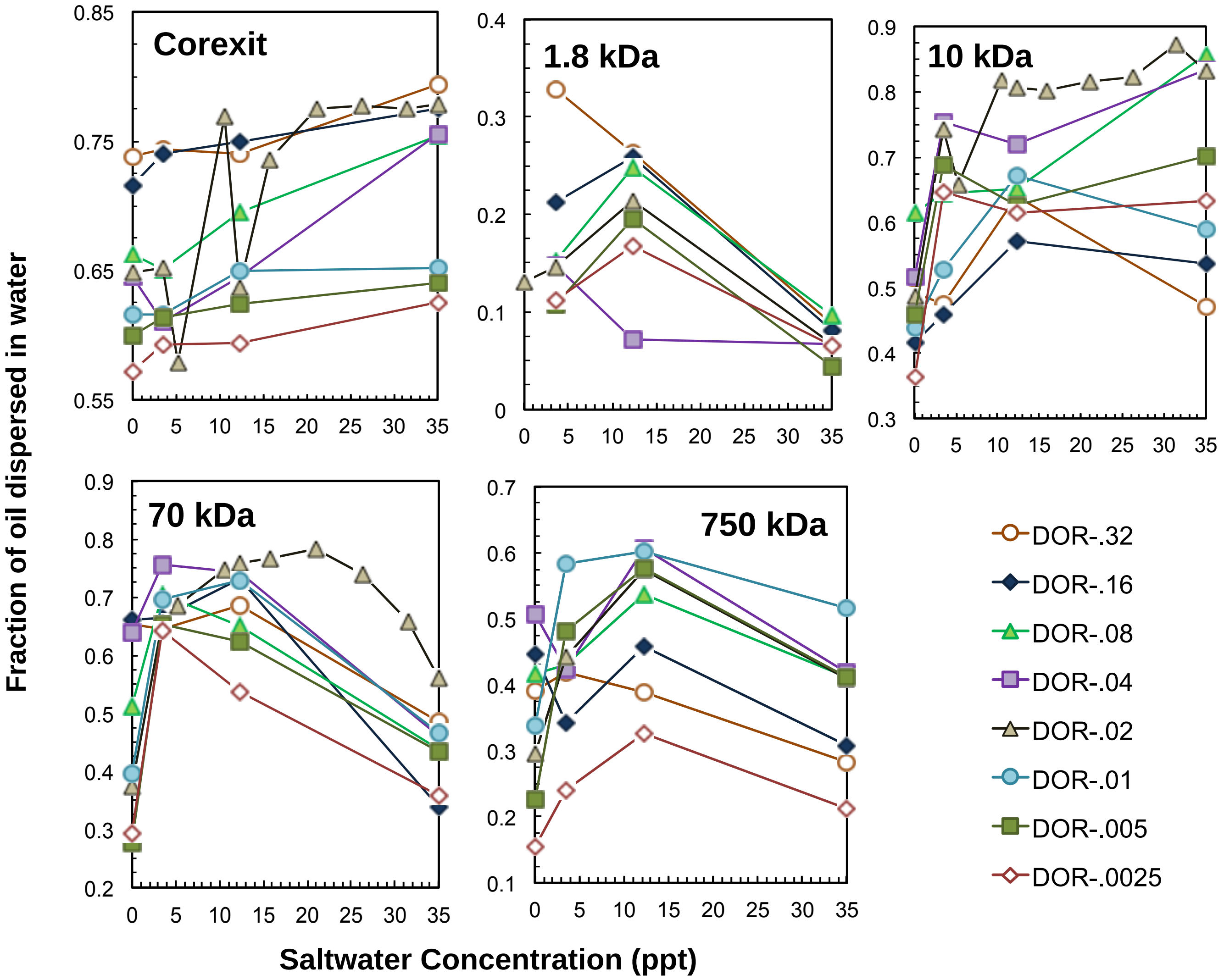

Figure 4-7. Total dispersion effectiveness data for Hy-PEI polymers and Corexit versus the fraction of artificial seawater used during effectiveness test at a range of DOR's. Note the different ranges on the y-axes.

4.2.3 pH Variation

The starting pH of the artificial seawater was varied with 1 M NaOH and 1 M HCl before dispersion tests. This study would show if the starting pH of the water had an affect on dispersion effectiveness. The starting pH was ranged from 5-11and these results can be seen in **Figure 4-7.**

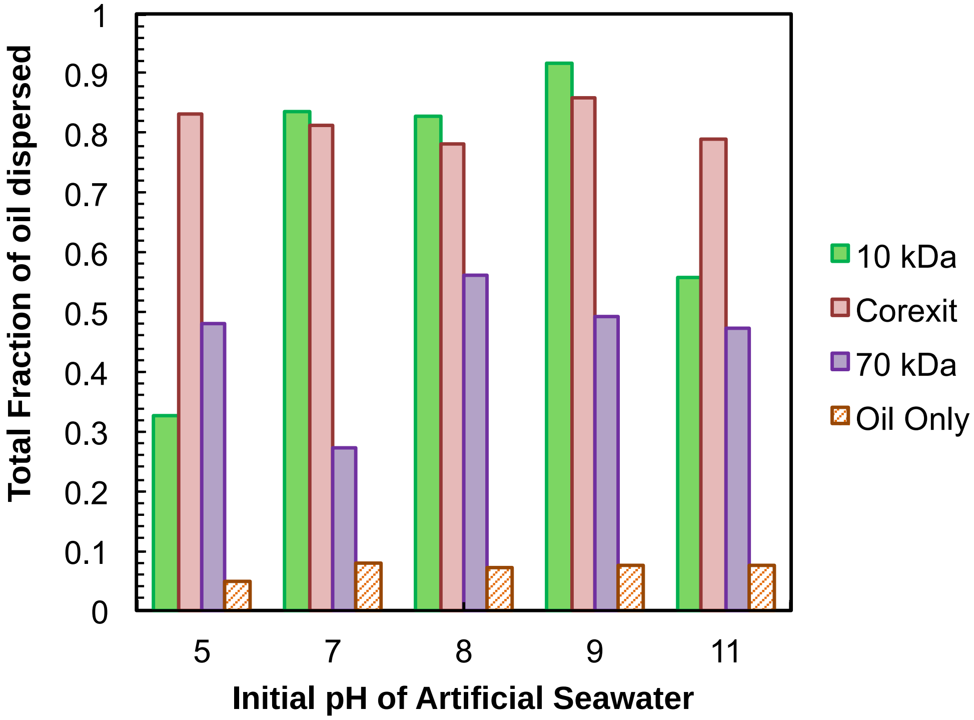

Figure 4-8. The initial pH of the artificial seawater for each sample is on the x-axis and its corresponding total fraction (sum of four layers) of oil dispersed is on the y-axis. The DOR for each experiment was 1:50 (0.02) and the saltwater concentration was 35 ppt.

The starting pH did not appear to influence the dispersion effectiveness of Corexit, oil only, or 70 kDa. However, at the more extreme pH's (5 and 11), 10 kDa experienced a decrease in dispersion effectiveness. This can be attributed to the change in concentration of H^+ ions due to the varying pH. As the pH decreases, more H^+ ions are present in solution. As the H^+ ion concentration increases, the negative surface charges of the oil droplets begin to become more neutral and the positive end groups of the Hy-PEI polymers become more highly charged. Conversely, as the pH increases, less H^+ ions are present in solution. This causes the oil droplet charge to become more neutral and the positive polymers to become more charged.

These changes in charge at extreme pHs decrease the attraction between the Hy-PEI polymers and oil droplets; however, when the solution is near neutral pH, both the oil and polymer surfaces retain their original, opposite charges causing the two to be attracted to each other and allowing the polymers to effectively disperse and stabilize the oil droplets. The mechanism that 10 kDa uses to disperse oil depends in part on the polymer binding to the oil droplets, which requires an attraction between the oil and polymer due to their opposite charges.

70 kDa did not have the expected, same trend at extreme pH's as 10 kDa. This could be because 70 kDa had less dispersion effectiveness overall. It could also be due to the larger size of 70 kDa. This size gives 70 kDa more sites for oil entrapment than 10 kDa. Therefore, this entrapment mechanism may be more important in determining the dispersion effectiveness for 70 kDa than the attraction from opposite surface charges.

The main mechanism that Corexit relies on to disperse oil is a surfactant mechanism. This is a lipid-lipid interaction when the hydrophobic tails of the surfactants are inserted into oil droplets regardless of surface charges. This does not depend on the attraction binding that the Hy-PEI 10 kDa polymer depends upon. Thus, the dispersion effectiveness of Corexit is independent of water pH.

In order to determine the effects dispersants have on water pH the artificial seawater was made with an initial pH of 8. The final pH of solutions with increasing DOR's of Corexit and the 10 kDa polymer were taken after the settling step of the effectiveness tests. As shown in **Figure 4-8**, dispersion from Corexit appeared to have little to no effect on the final pH, but as the DOR of the 10 kDa was increased, the pH of water also increased.

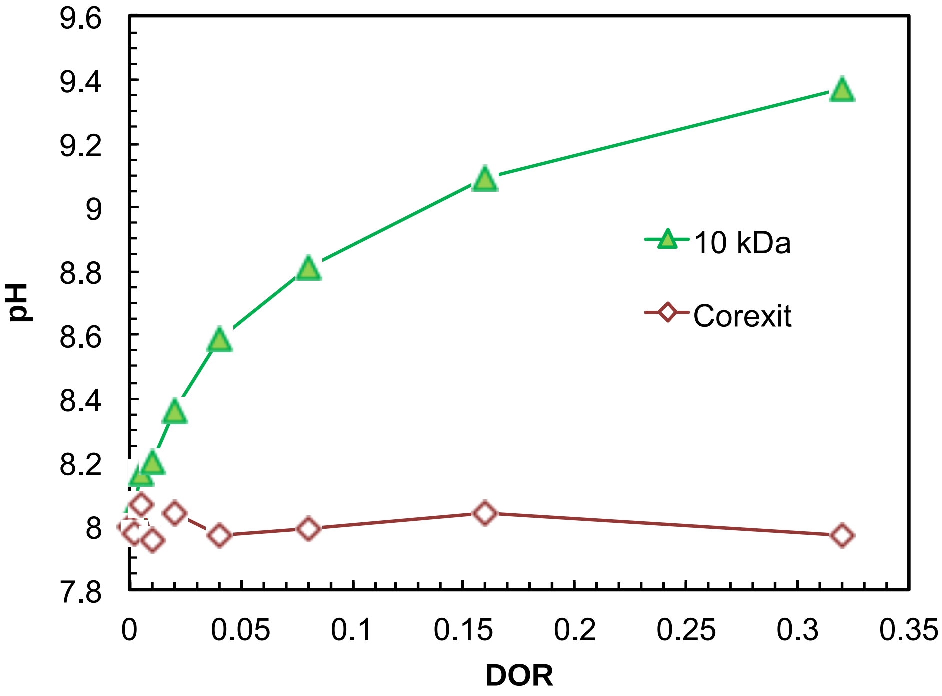

Figure 4-9. pH changes of artificial seawater after dispersion effectiveness tests were run with various DOR's of Corexit and the 10 kDa Hy-PEI polymer. The initial pH of 8 is marked as the DOR of 0.

The pH of the water increasing implies that the Hy-PEI polymers must be taking protons out of the water. The amine terminal groups can take up protons and affect the pH. The artificial seawater's initial pH of 8 is lower than the ~10.6 pKa of the amines. Therefore, protons are taken up, thus increasing the pH of the water. The more polymer that is present (higher DOR), the more protons are taken up, and the higher the pH increases.

The basicity of Hy-PEI polymers should not be a large concern for wide spread use because dispersants are most often applied to oil slicks at an average DOR of 1:20 (0.05) [26]. At this low DOR, the pH rise is minimal. In addition, an increase in pH would be localized to the point of entry of the polymers; the effect would be quickly diluted in the ocean system.

4.2.4 Combined Effects

In order to evaluate various effects in combination with one another, the program JMP was used to evaluate all of the collected data. The matrix generated can be seen in **Appendix B**. This matrix allows the reader to go to a specific scenario (salinity, DOR) and compare dispersant materials and also allowed to observe any additional synergies between DOR and salinity that were affecting a specific dispersant's performance. Each of these graphs supported the data collected previously; Corexit appears to behave better at higher salinities and DOR's, whereas the polymers can still perform in lower salinities, but are experience a decrease in effectiveness at higher DOR's and salinities.

4.3 Oil Droplet Size

The microscope images of oil droplets dispersed in various artificial saltwater and DOR solutions can be seen in **Figure 4-9.**

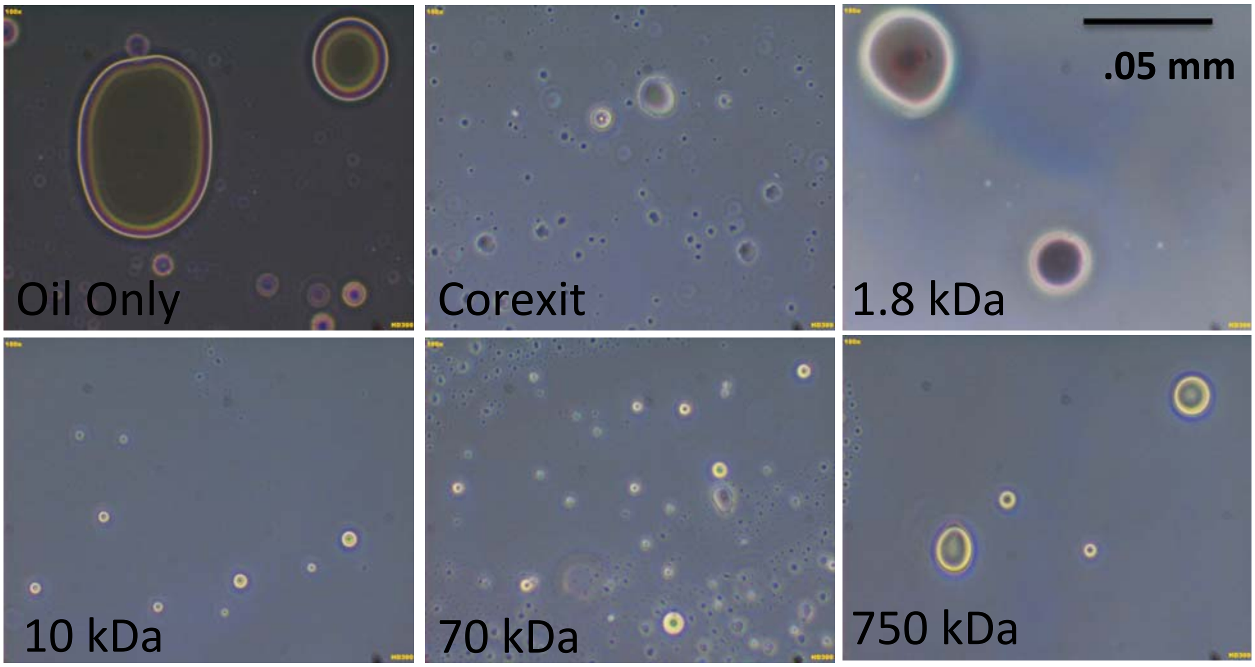

Figure 4-10. Visible light microscope images taken on phase contrast at 1000x magnification. Oil droplets are suspended in 100% artificial seawater; any dispersant added was added at a 1:50 (0.02) DOR.

The droplet sizes appear to follow the same trends as the dispersion effectiveness tests. A larger droplet size will result in less dispersion because larger drops are less stable and more likely to aggregate until they are large enough to rise out of the water column. **Figure 4-9** shows the biggest oil droplets when the oil is subjected to artificial seawater with no dispersant and with 1.8 kDa. The oil droplets then appear to be much smaller in solutions with 10 kDa, Corexit, and 70 kDa; with slightly larger droplets with 750 kDa. This accurately follows increasing dispersion effectiveness of oil only, 1.8 kDa, 750 kDa, 70 kDa, Corexit, and 10 kDa.

It should be noted that the halos surrounding the oil droplets indicate that the droplets are not fully in focus. This distorts the image and may not represent a truly accurate droplet size. Because there were so many planes of focus, these halos were impossible to avoid when using a microscope to capture the images; thus these data are qualitative and could not be used to quantitatively determine the drop size.

4.4 Interfacial Tension Measurements

As done in Tu's work [51], the Kruss Easydrop instrument was used to determine the IFT between oil droplets and water. Shown in **Figure 4-10**, the preliminary results with only oil (no dispersant) in artificial freshwater and artificial seawater indicate that the presence of ions in the seawater drops the IFT between the oil droplets and the water.

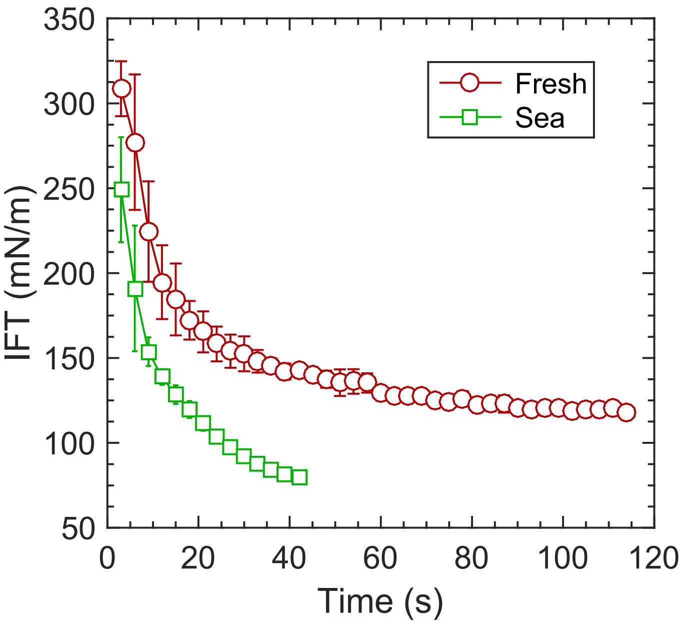

Figure 4-11. IFT measurements of a crude oil drop in artificial freshwater and artificial seawater versus time. There was no dispersant added to this water. The error bars indicate the standard deviation for three separate runs. Absent error bars are smaller than the symbols.

The same trend of IFT drop-off in artificial seawater versus artificial freshwater was observed when Corexit was added to the artificial waters for experimentation, **Figure 4-11**. Also, as the dose of Corexit present in the waters increased, the IFT dropped. This was consistent with the dispersion effectiveness tests that Corexit had better effectiveness in higher saltwater concentrations and higher DOR's. The main mechanism that Corexit works through is the surfactants present, which decrease the IFT between oil and water; which is reflected in the IFT measurements.

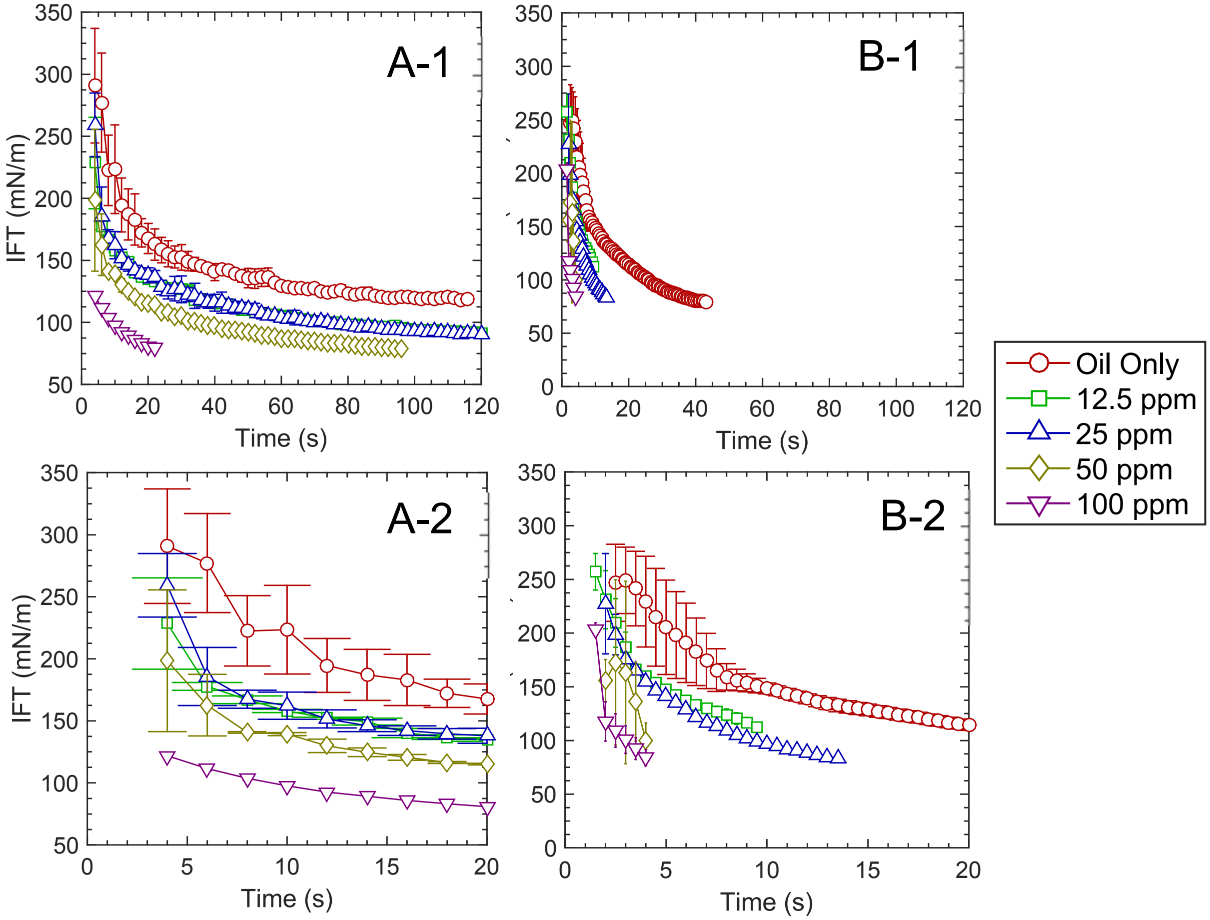

Figure 4-12. IFT between a 3-μL oil droplet and (A) artificial freshwater or (B) artificial seawater. The artificial waters were dosed with various amount of Corexit to get a DOR of 12.5-100 ppm. Error bars indicate the standard deviation for three separate runs. Error bars not seen are smaller than the symbol. A-2 and B-2 have the same data as A-1 and B-2 respectively on a smaller x-axis scale to better observe the differences in IFT of dispersant dosages.

Figure 4-11 and **Figure 4-12** show the comparison of each dispersant's IFT measurements at 12.5 ppm in artificial fresh and seawater. In the artificial seawater, the polymers showed the same trends as shown in dispersion effectiveness. However, Corexit did not appear to reduce the surface tension as much as was expected based on the dispersion trends. This could be because when Corexit is mixed in water alone, the surfactants may have interacted with water, limiting their interaction with the oil droplet; this behavior is even further evident in the artificial freshwater. This demonstrates the importance of applying Corexit directly to the oil slick, and not to the water.

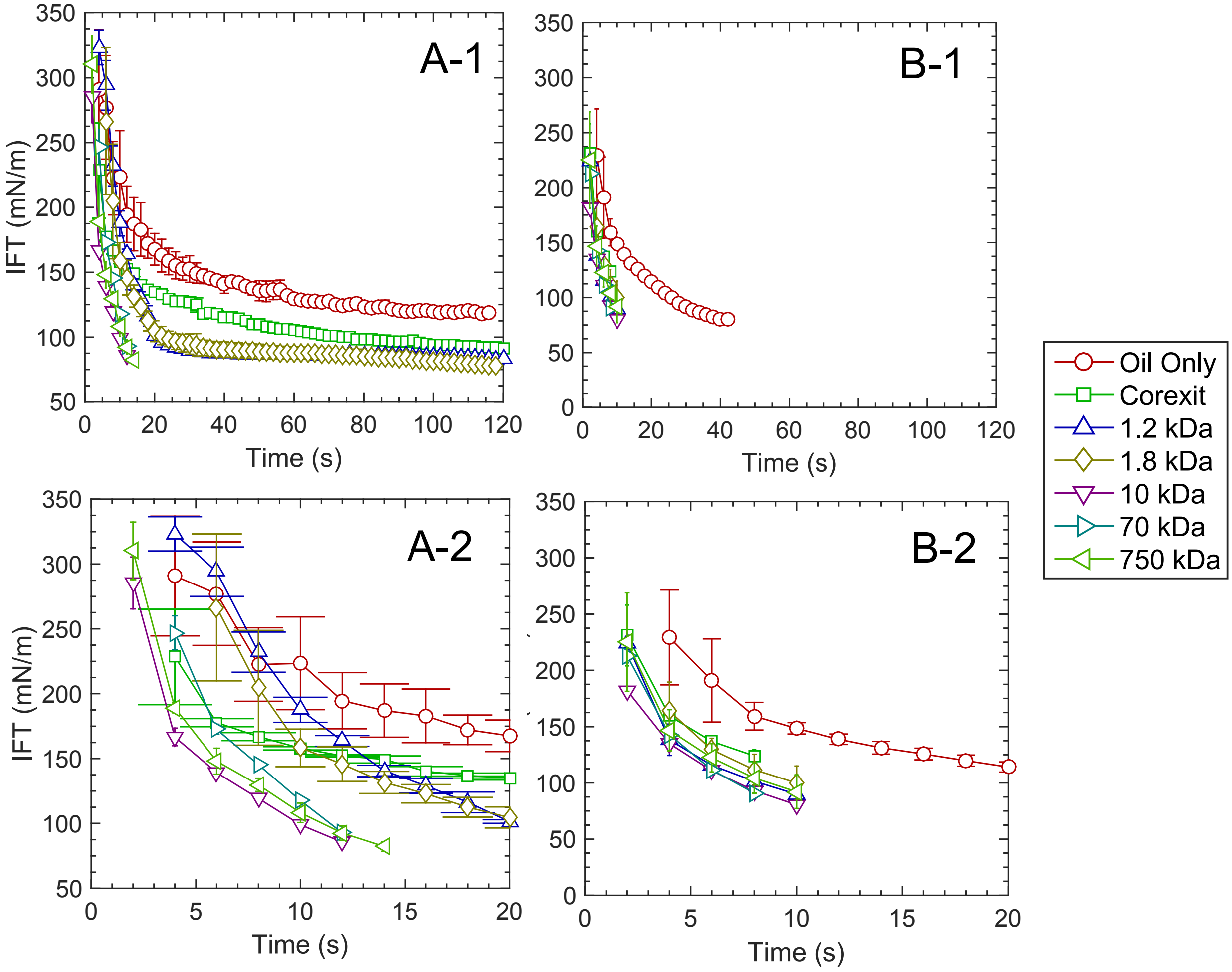

Figure 4-13. IFT measurements of a crude oil drop in (A) artificial freshwater and (B) artificial seawater versus time. Oil only indicates no dispersant added, all other dispersants were added at a dose of 12.5 ppm. A-2 and B-2 have the same data as A-1 and B-2 respectively on a smaller x-axis scale to better observe the differences in IFT of dispersant dosages. The error bars indicate the standard deviation for three separate runs. Error bars not seen are smaller than the symbol.

In addition to Corexit not following the dispersion effectiveness trends, 1.2 and 1.8 kDa did not have the expected effect on IFT either. Based on the dispersion effectiveness results alone, it was presumed that 1.2 and 1.8 kDa would have little to no effect on lowering the IFT between the oil droplet and water. However, both of these polymers did lower the surface tension significantly. This may be because the polymers behave differently than surfactants in oil dispersion. With Corexit, the reduction of surface tension is the main mechanism in which the oil is dispersed, but polymers employ both surface tension reduction and oil entrapment via hydrocarbon bridging. The unexpected trends in the IFT measurements point to the importance of entrapment for a polymer to be able to disperse oil adequately. Though 1.2 and 1.8 kDa lowered the surface tension of the oil, this was not adequate for the polymers to display substantial dispersion effectiveness.

Consistently, there is more variability (higher standard deviation) in the earlier time IFT measurements. In large part, this is due to the fact that the drop is not very stable at the earlier times (the drop is still forming). Once the drop is allowed to completely form, the standard deviation drops significantly.

4.5 Hy-PE-PEG Polymer Effectiveness

Figure 4-13 shows the dispersion effectiveness results of the Hy-PE-PEG polymers in 35 ppt salinity and a 1:50 DOR. **Figure 4-14** compares the total effective off the Hy-PE-PEG polymers to the total effectiveness of the Hy-PEI polymers and Corexit.

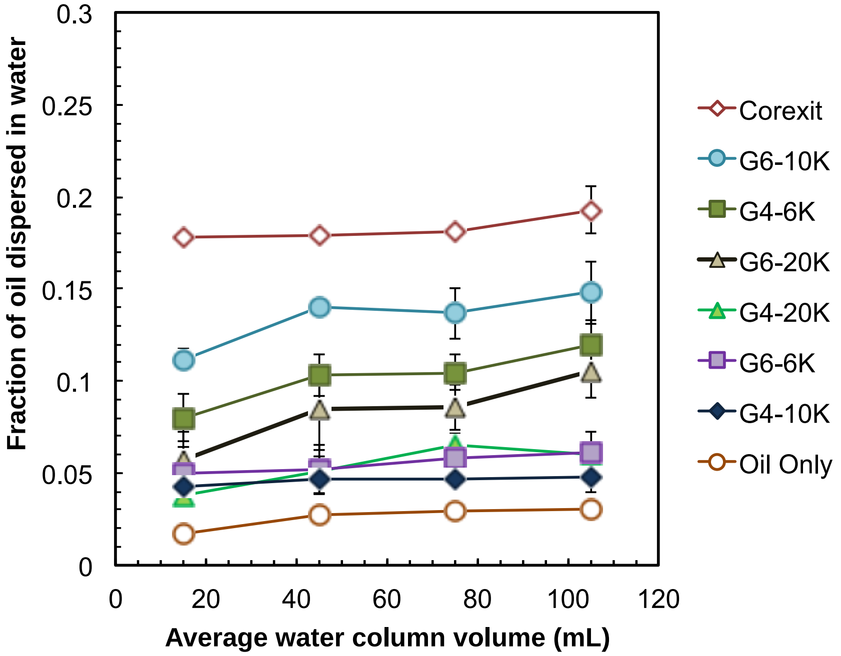

Figure 4-14. Oil dispersion effectiveness data for Hy-PE-PEG polymers compared with Corexit and a crude oil control (no dispersant) in artificial seawater. Error bars represent the standard deviations from three distinct experiments. Absent error bars are smaller than the symbols.

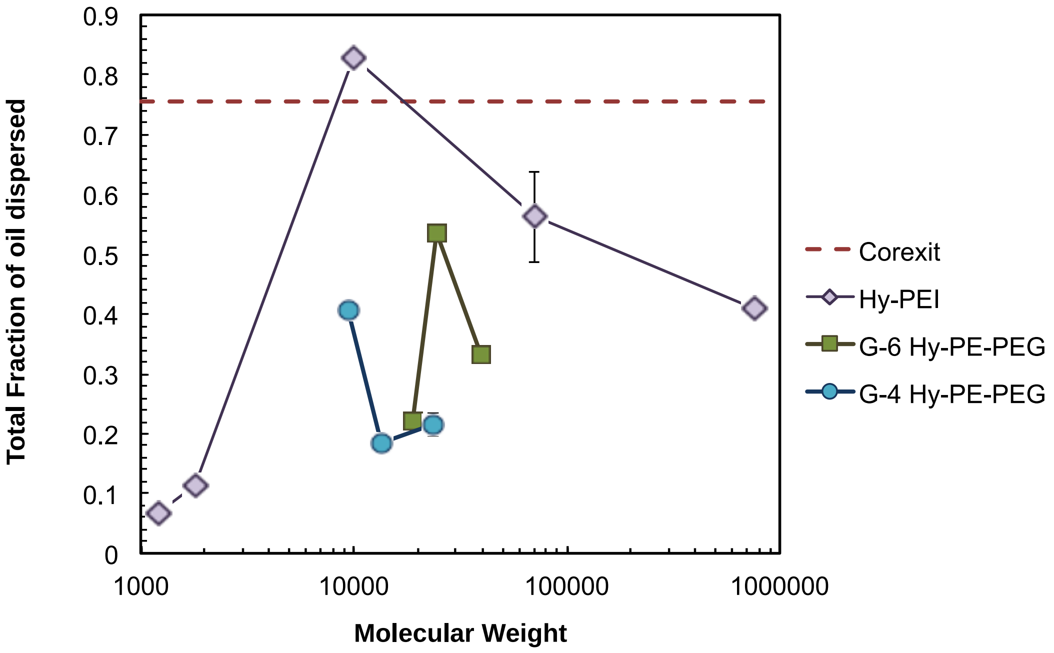

Figure 4-15. Comparison of Hy-PEI and Hy-PE-PEG polymers' total dispersion effectiveness versus the polymer's molecular weights. The DOR for all polymers is 1:50 (0.02) and done in 100% artificial seawater.

As seen in **Figure 4-13** and **Figure 4-14** there were no Hy-PE-PEG polymers that worked as well as Corexit at a 1:50 (0.02) DOR in artificial seawater. More than likely this is due to the charge of the Hy-PE-PEG polymers. Though the neutral charge is better in terms of biodegradability, it appears to have the expected but unfortunate impact of lowering a dispersant's effectiveness. This is because the crude oil has an overall negative charge. Thus the positively charged end groups on the Hy-PEI polymers are able to attract the oil to the dispersant, allowing for higher dispersion. Whereas the netural charged end groups lack this attractive force to the oil, which decreases the polymer's ability to attach to and disperse the oil droplets.

Unlike the Hy-PEI polymers, the Hy-PE-PEG polymers did not appear to exhibit a clear trend between molecular weight and dispersion effectiveness for the Hy-PE-PEG polymers, shown in **Figure 4-14**. Neither generation 4 nor generation 6 of the Hy-PE-PEG polymers appeared to have a correlation with dispersion effectiveness either.

4.5.1 Hy-PE-PEG Effectiveness in Artificial Freshwater

The Hy-PE-PEG polymers were also tested for dispersion effectiveness in artificial freshwater. These results can be seen in **Figure 4-15**. The polymers showed no capability for dispersion in the artificial freshwater. The comparison of the Hy-PE-PEG effectiveness in artificial saltwater and freshwater is shown in **Figure 4-16**. The few Hy-PE-PEG's that had little dispersion capability in the saltwater, had no capability in freshwater. This can be accounted for because the saltwater compressed the double layers of the Hy-PE-PEG polymers and oil and there was less repulsion between the two, to allow for slight dispersion.

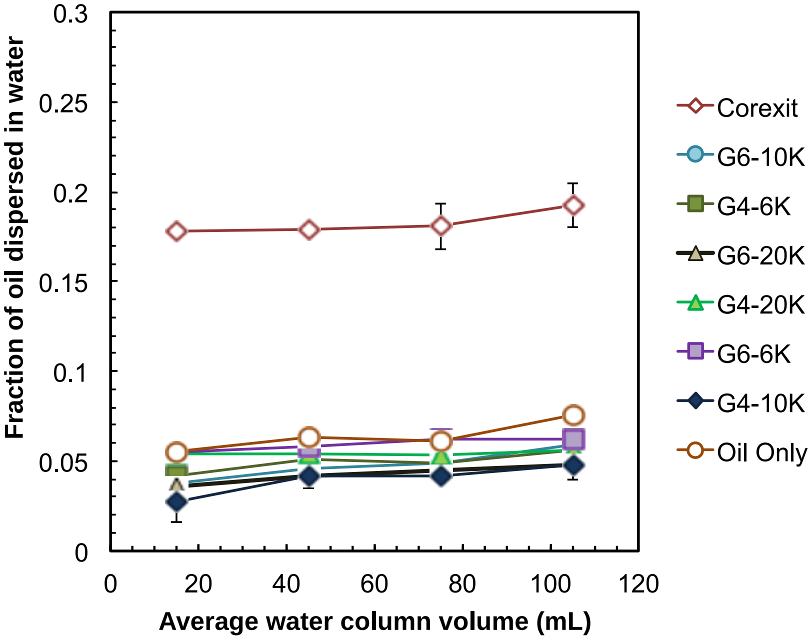

Figure 4-16. Oil dispersion effectiveness data for Hy-PE-PEG polymers compared with Corexit and a crude oil control (no dispersant) in artificial freshwater. Error bars represent the standard deviations from three distinct experiments. Absent error bars are smaller than the symbols.

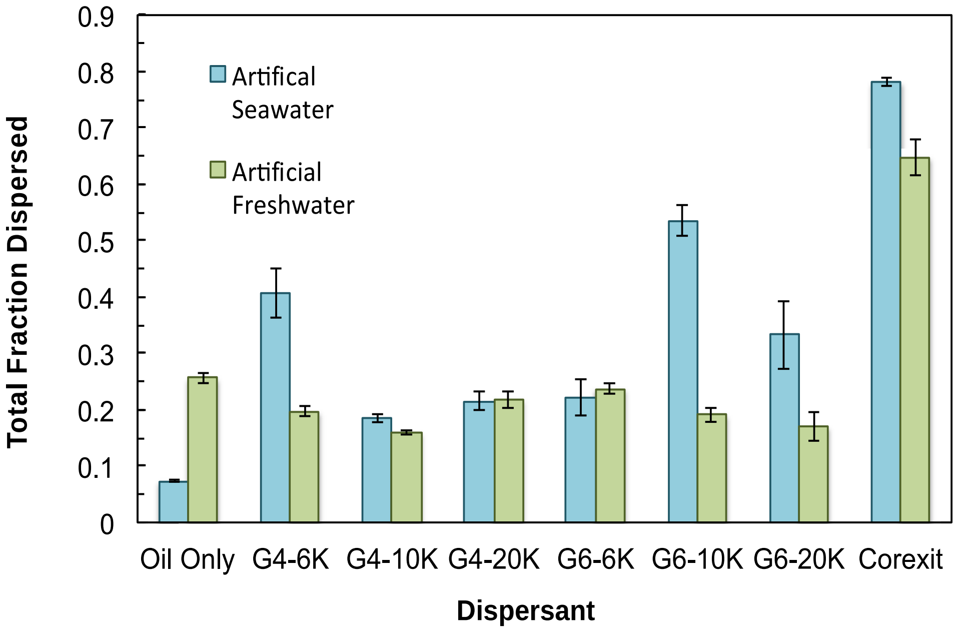

Figure 4-17. Comparison of the total dispersion effectiveness for Hy-PE-PEG polymers and Corexit in both artificial seawater and artificial freshwater. Error bars represent the standard deviations from three distinct experiments.

4.5.2 Hy-PE-PEG IFT Measurements

Figure 4-17 and **Figure 4-18** show the IFT measurements taken between an oil droplet and waters dosed with the Hy-PE-PEG polymers. Consistent with the dispersion effectiveness results, the Hy-PE-PEG polymers showed no reduction in IFT measurements when compared with the oil only control in both artificial freshwater and seawater. As with the Hy-PEI polymers, the oil droplet had a lower IFT in artificial saltwater than in artificial freshwater. These results were the same when the water was dosed with 12.5 ppm and 100 ppm of the Hy-PE-PEG polymer. Since both of these polymer concentrations yielded practically identical IFT measurements, it was unnecessary to test the mid-ranged dosages (25 and 50 ppm).

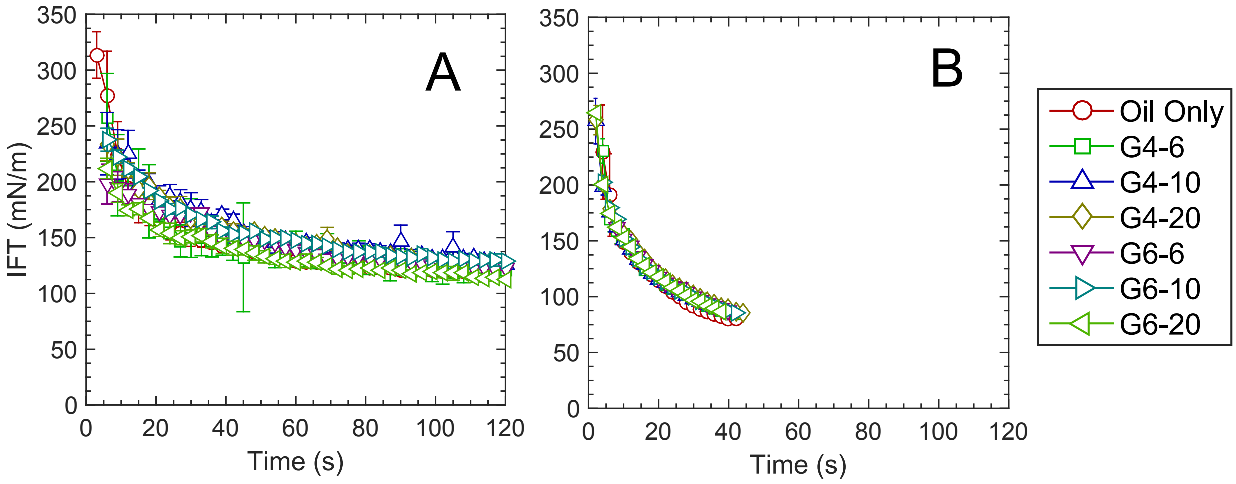

Figure 4-18. IFT between a 3-µL oil droplet and (A) artificial freshwater or (B) artificial seawater. The artificial waters were dosed with a DOR 12.5 ppm for each Hy-PE-PEG polymer.

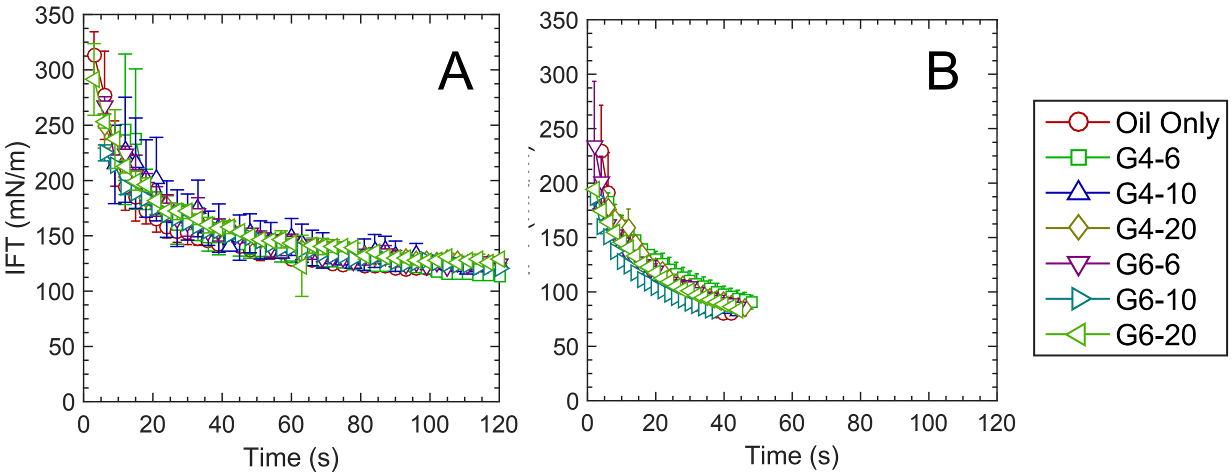

Figure 4-19. IFT between a 3-μL oil droplet and (A) artificial freshwater or (B) artificial seawater. The artificial waters were dosed with a DOR 100 ppm for each Hy-PE-PEG polymer.

4.6 Baffled Flask Tests of Hy-PEI Polymers

After the fraction of oil dispersed for each sample was calculated, the results were graphed versus the molecular weight of the Hy-PEI polymers alongside the data of total fraction dispersed versus molecular weight obtained by Tu's protocol. The results shown in **Figure 4-20** clearly show the similarities of the two tests.

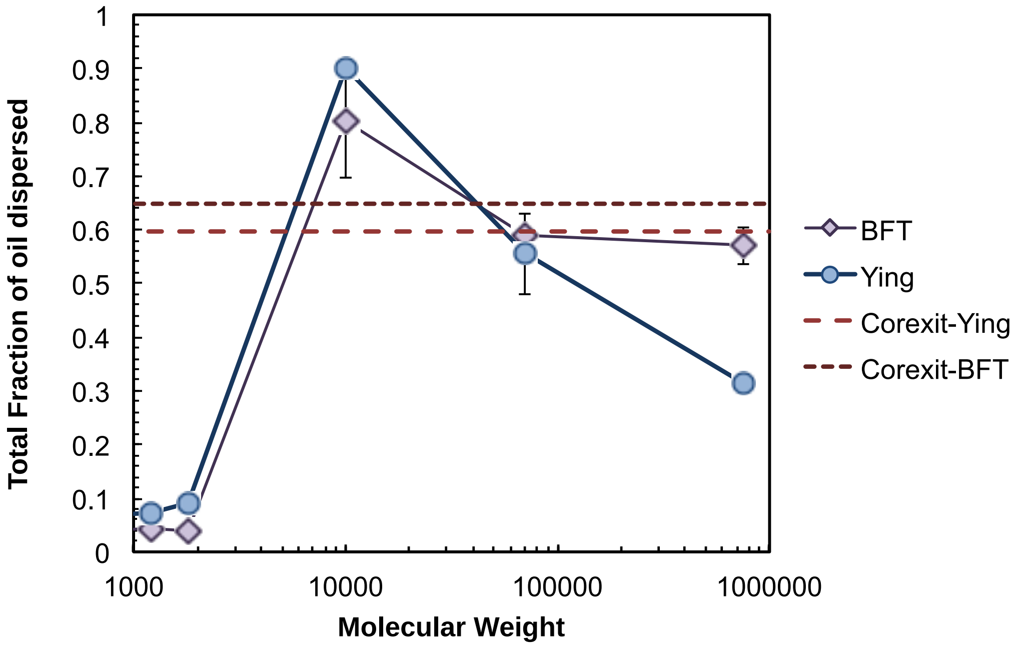

Figure 4-20. Comparison of dispersion effectiveness for the Hy-PEI determined by the BFT protocol and Tu's protocol versus they polymer's molecular weights. The DOR for BFT is 1:25 (0.04), the DOR for Tu's protocol is 1:50 (0.02); both were run in 100% artificial seawater. Error bars represent the standard deviation from two runs for the BFT and three runs for Tu's protocol. Error bars that cannot be seen are smaller than the symbol.

The main goal of these experiments was to view the relationships in dispersion effectiveness between various oil dispersants. Absolute values are less valuable because of the extremely different conditions that dispersants will experience in the real world; absolute values are less likely to correspond in real-world applications than the trends. Once the BFT was developed, it underwent various methods of testing. Afterwards, many researchers and the EPA agreed that the BFT was more accurate than previous protocols and it was labeled as the standard method for determining a dispersant's dispersion effectiveness. Since the EPA has chosen a standardized test, it was necessary to check that the trends seen in Tu's dispersion tests had the same dispersant relationships to that of the BFT. Not only were the relationships very similar, but the absolute values were also comparable. It was expected that the relationships of dispersion effectiveness between the various oil dispersants would be confirmed with the BFT, but since the two protocols differed on mixing, extraction, calibration, and calculation techniques it was a slight surprise that the absolute values from the two experiments yielded such similar results. From these results, it can be concluded that the dispersion technique developed by Tu is as accurate at determining the effectiveness of dispersants as the BFT in both dispersant comparison and absolute values.

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 Assessment of Objectives

(1) ***Determine which Hy-PEI polymers have the highest effectiveness.*** Based on a DOR of 1:50 in (0.02) 35 ppt artificial seawater, 10 kDa is the Hy-PEI polymer that has the highest effectiveness (82.9%). This is also the only polymer under these conditions that performed better than Corexit (78.1%). 1.2 and 1.8 kDa had little to no dispersion capabilities whereas 70 and 750 kDa began to interact and cause a decrease in effectiveness compared to the 10 kDa.

(2) ***Examine how varying dispersant concentration influences dispersant effectiveness.*** As Corexit's DOR is increased, the effectiveness increases. However, 70 and 750 kDa saw a decrease in effectiveness after a peak at the 1:25 (0.04) DOR and 10 kDa experienced this decrease a 0.16 DOR. This could be due to interactions of the polymers causing agglomeration and oil coalescence.

(3) ***Examine how varying water characteristics influences dispersant effectiveness.*** The increase of saltwater concentration had the most influence on dispersant effectiveness. Whereas Corexit had a greater effectiveness with increasing saltwater concentration, 70 and 750 kDa had interactions with the increasing ions in the artificial seawater that began to inhibit their dispersion ability.

(4) ***Examine any synergies between dispersant concentration and water characteristics on dispersant effectiveness.*** The main synergy observed was with DOR and saltwater concentrations, especially with the higher molecular weighted

polymers. Interactions between the polymers and the saltwater appeared to further hinder effectiveness as DOR and saltwater concentration increased. For real-world applications it is important to take note of all the effects that the water could have on the dispersant in order to determine the dispersant and dosage that has been shown to best disperse the oil in those aquatic conditions.

(5) ***Test the effectiveness of a novel set of HBP's with a polyester structure.*** The neutrally charged end group of the linear dendritic polymers significantly decreased the polymers' ability to disperse oil. No versions of the Hy-PE-PEG polymers were able to disperse oil as well as Corexit or the Hy-PEI polymers with higher molecular weights.

(6) ***Compare the test developed by Ying Tu to the EPA standardized Baffled Flasks Test (BFT).*** Results of both tests showed similar trends and similar absolute effectiveness values. It can be concluded that dispersant results from the effectiveness test developed by Tu will have very similar result if run by the BFT.

5.2 Recommended future studies

In order to get a quantitative measurement of oil droplet size, a new technique needs to be developed. The technique needs to be able to capture oil droplets that are totally in focus as well as analyze the images to get an accurate drop measurement. These data will allow the dispersants to be better studied.

For future research it would be important to elucidate whether the charge of the polymers is the most important property affecting dispersion or whether the backbone or core of the polymer is actually most important (or perhaps they are equally important). To do this identical Hy-PEI polymers (same structure and molecular weights) except with neutrally charged end groups should be tested for effectiveness. Likewise, Hy-PE-PEG polymers with positively charged end groups should be tested. In addition, these same polymers should be tested with negative terminal charges.

There are also many different aquatic parameters that were not tested with this research. Dispersion could also be significantly affected by temperature, mixing present, natural organic matter present, microbes present, etc. Additional experiments may explore varying these parameters to see their effects on a dispersant's effectiveness.

In addition, further toxicity studies should be done on the polymers to confirm the initial assumption that these polymers pose less of an environmental threat than Corexit. These studies should investigate the toxicity, environmental biocompatibility, biodegradability, and potential for bioaccumulation for any polymer being proposed for use as an oil dispersant.

Finally, for practical application of any novel dispersant, cost must be taken into account. HBPs were used here rather than dendrimers because HBP synthesis is much faster and cheaper than dendrimer synthesis. However, whether HBP synthesis is comparable in cost to conventional surfactants (like those used in Corexit) remains to be determined. Surfactants have a long history of mass-production for inclusion in many products; a similar scale of production would likely be needed to make the cost of HBPs competitive with surfactants.

APPENDICES

Appendix A – BFT Calibration Curves

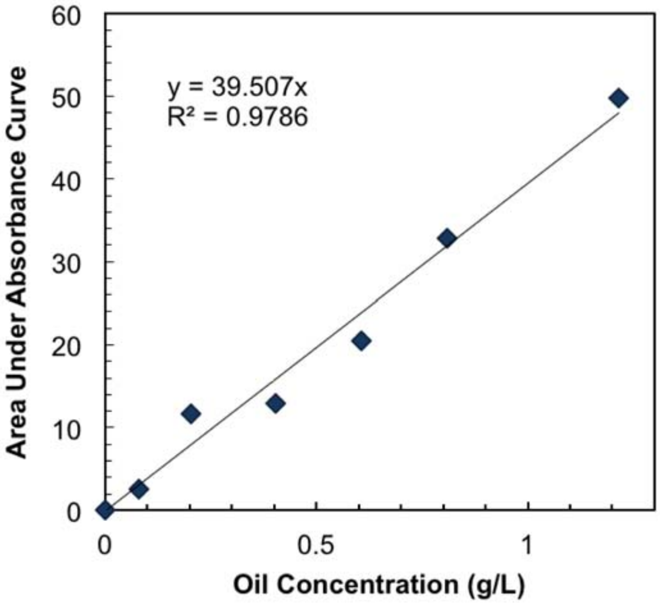

Figure A-1. Calibration curve from the BFT for oil only (no dispersant). The slope was used to calculate dispersion effectiveness.

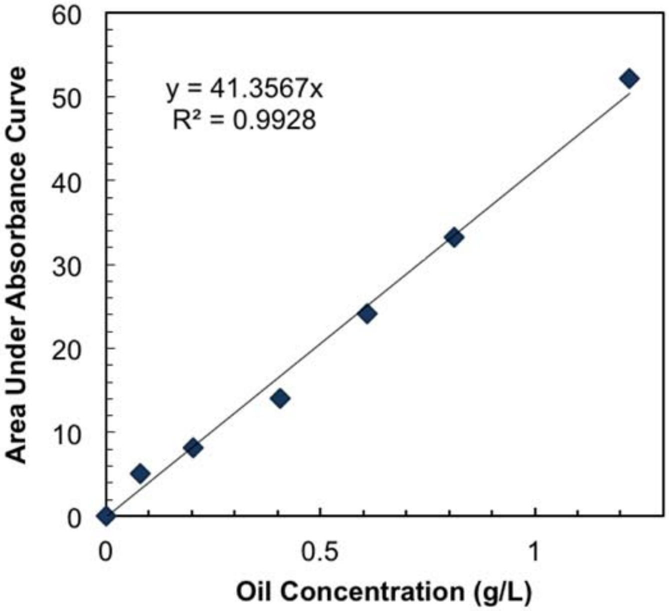

Figure A-2. Calibration curve from the BFT for oil and Corexit. The slope was used to calculate Corexit's dispersion effectiveness.

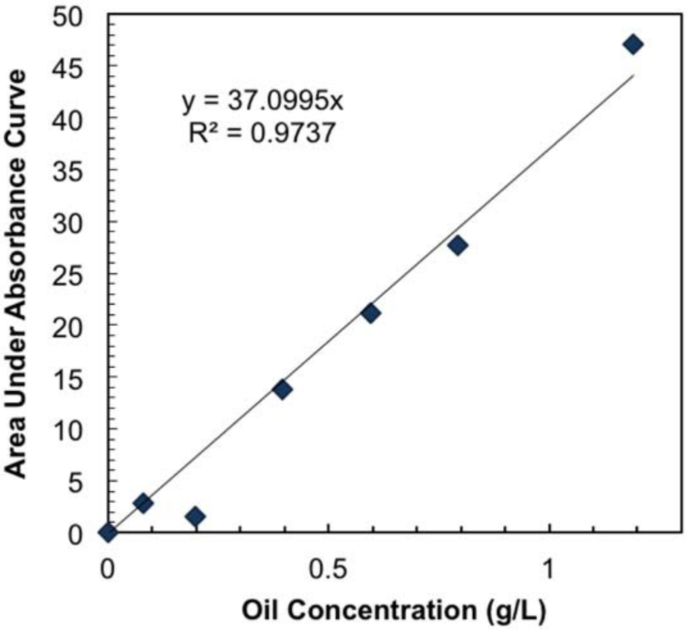

Figure A-3. Calibration curve from the BFT for oil and 1.2 kDa. The slope was used to calculate 1.2 kDa's dispersion effectiveness.

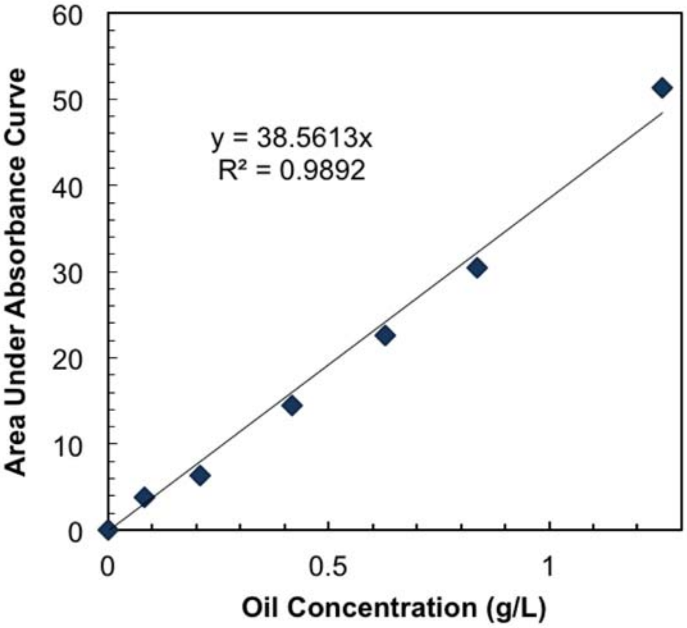

Figure A-4. Calibration curve from the BFT for oil and 1.8 kDa. The slope was used to calculate 1.8 kDa's dispersion effectiveness.

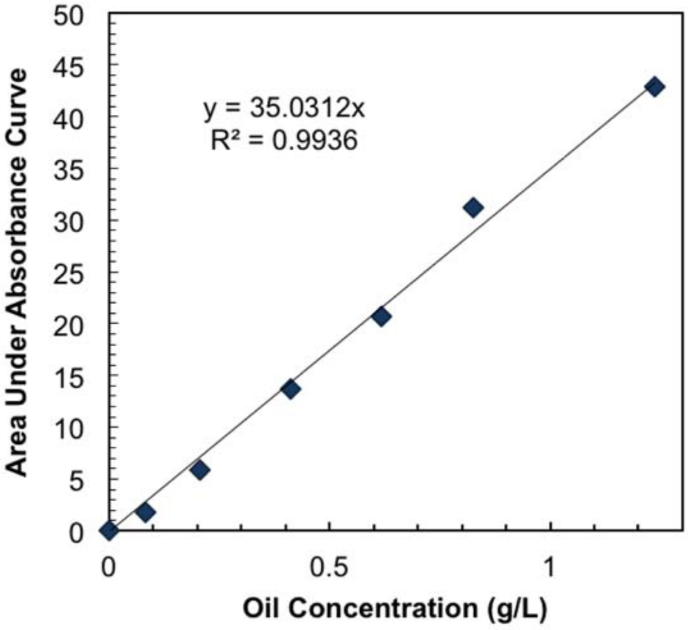

Figure A-5. Calibration curve from the BFT for oil and 10 kDa. The slope was used to calculate 10 kDa's dispersion effectiveness.

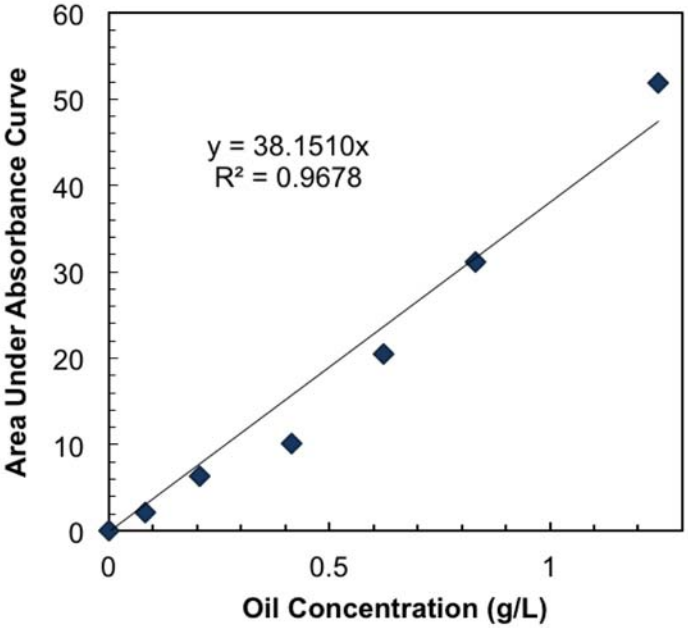

Figure A-6. Calibration curve from the BFT for oil and 70 kDa. The slope was used to calculate 70 kDa's dispersion effectiveness.

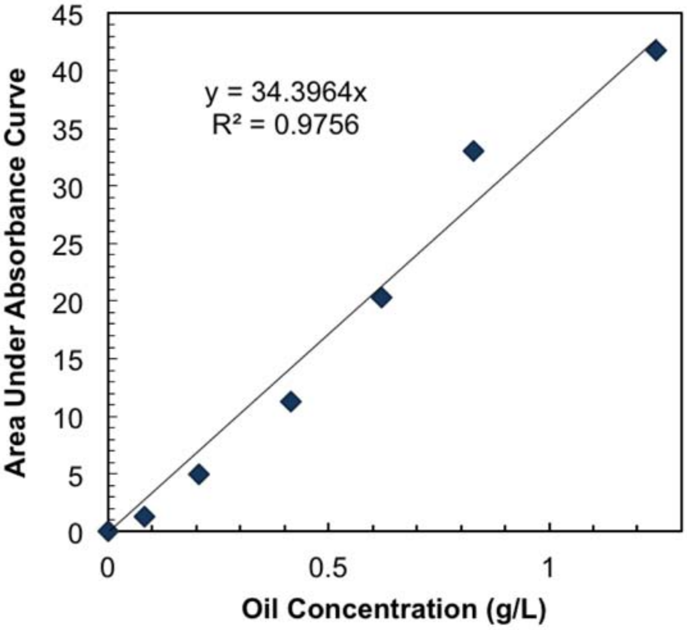

Figure A-7. Calibration curve from the BFT for oil and 750 kDa. The slope was used to calculate 750 kDa's dispersion effectiveness.

Appendix B – JMP Graphs

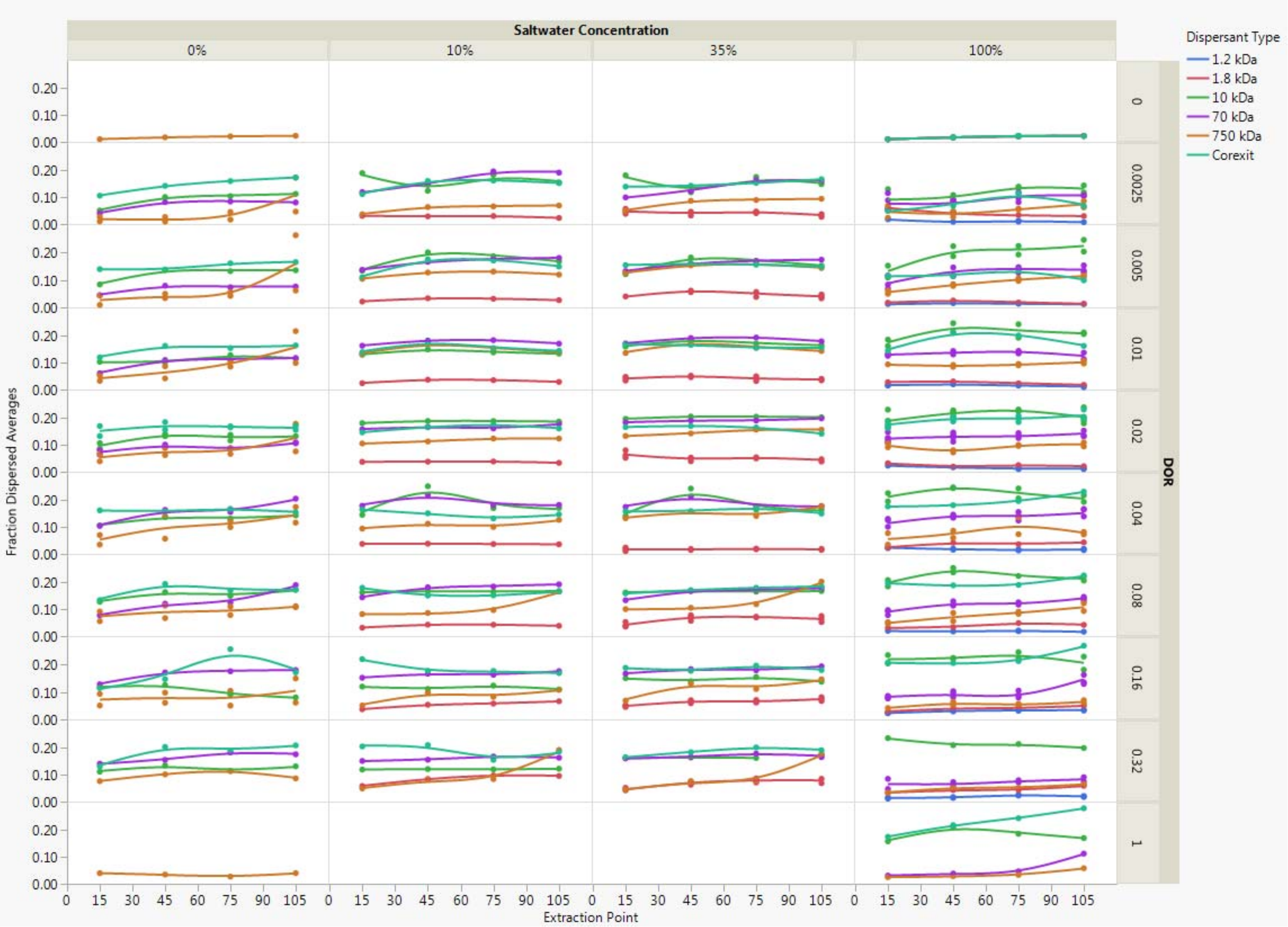

Figure B-1. Fraction of oil dispersed at four extraction points in a matrix of various DOR's and saltwater concentrations for each dispersant. A way to determine which dispersant has the most effective behavior in a specific scenario.

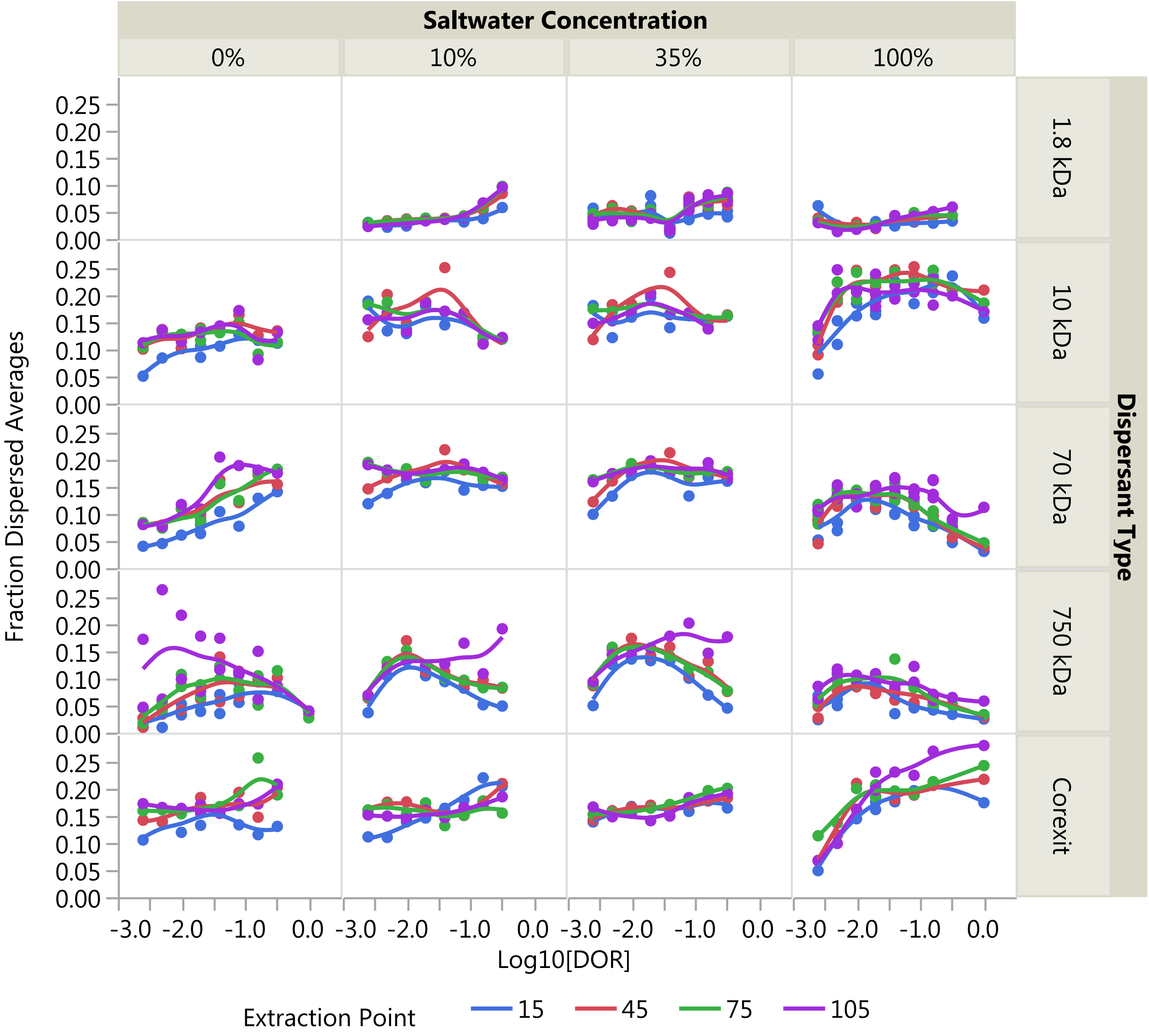

Figure B-2. Fraction of oil dispersed versus DOR in a matrix of saltwater concentration and dispersant type for each of the four extraction points. A way to determine the overall trend of increasing DOR for each dispersant in the various saltwater environments.

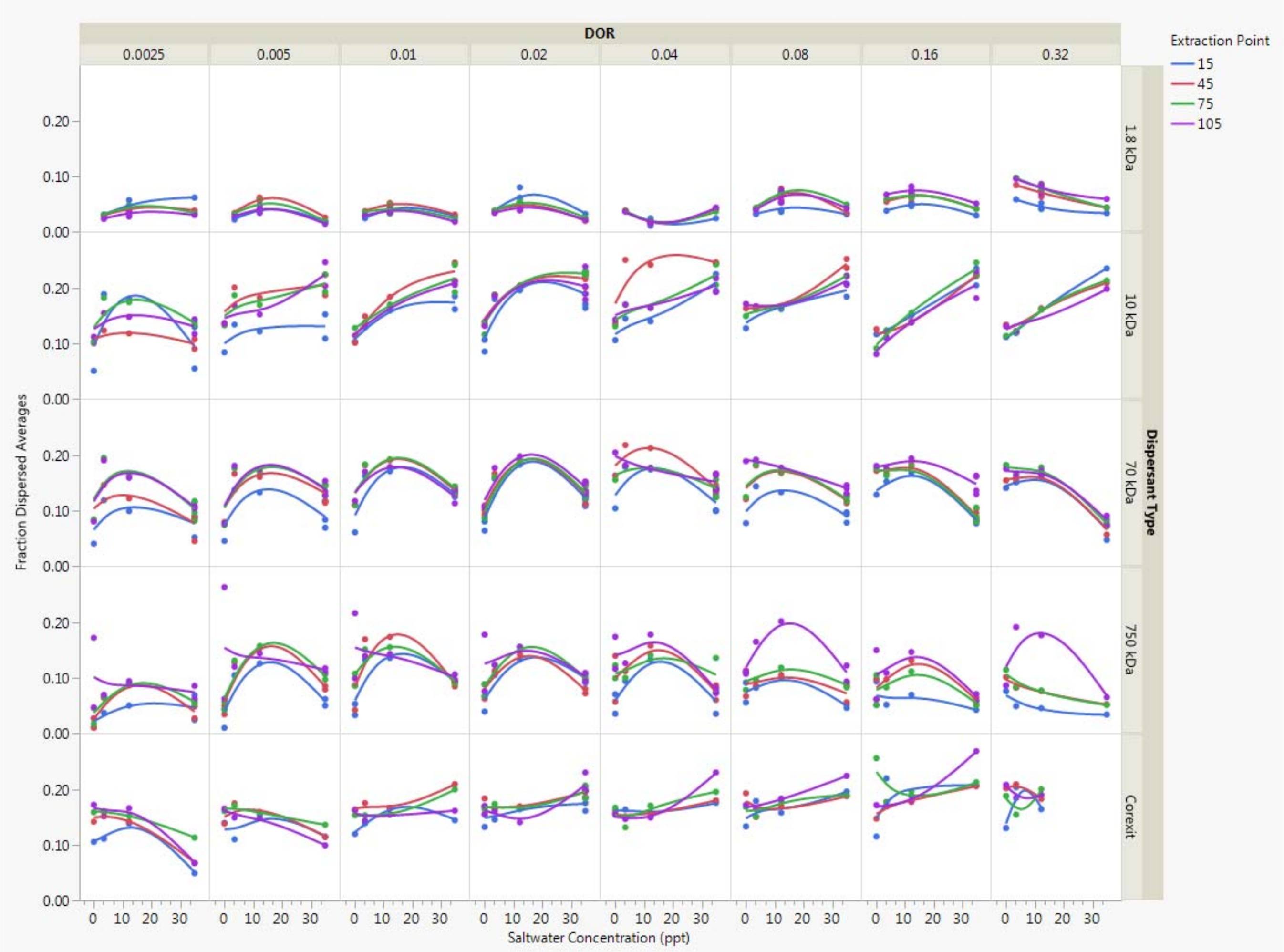

Figure B-3. Fraction of oil dispersed versus saltwater concentration in a matrix of DOR and dispersant type for each of the four extraction points. A way to determine the overall trend of increasing saltwater concentration for each dispersant for the various DOR's.

Appendix C – IFT Graphs

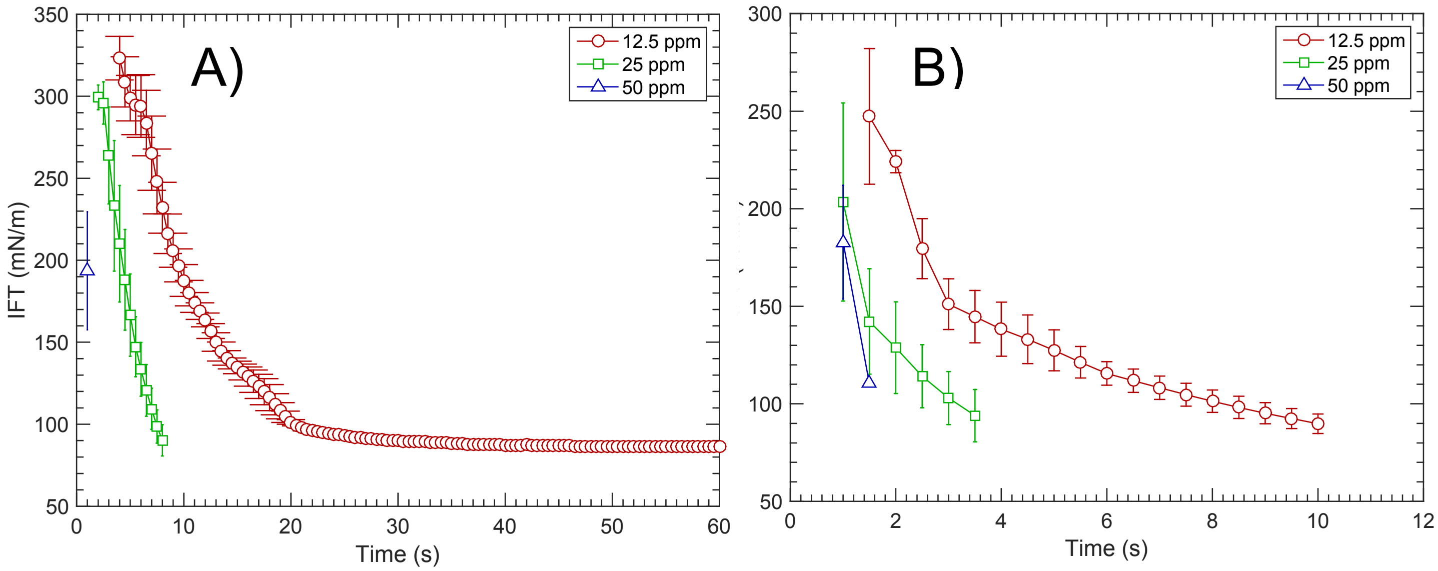

Figure C-1. IFT between a 3-µL oil droplet and (A) artificial freshwater or (B) artificial seawater (B). The artificial waters were dosed with various amount of 1.2 kDa to get a DOR of 12.5-100 ppm.

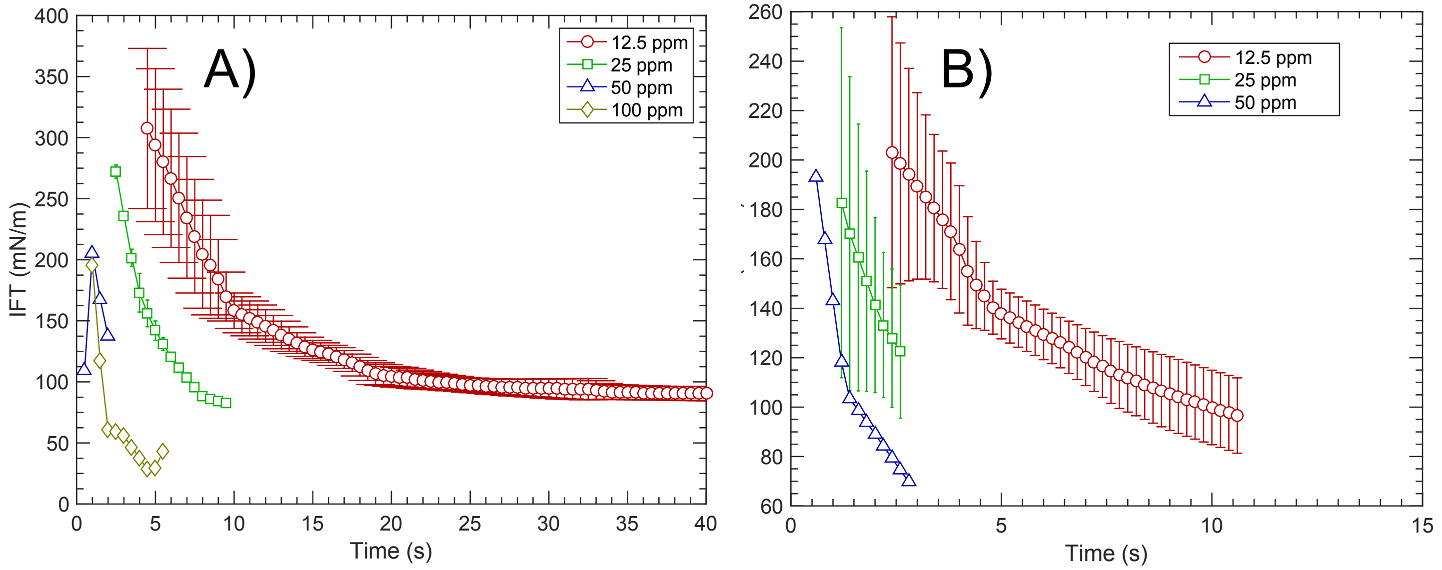

Figure C-2. IFT between a 3-µL oil droplet and (A) artificial freshwater or (B) artificial seawater (B). The artificial waters were dosed with various amount of 1.8 kDa to get a DOR of 12.5-100 ppm.

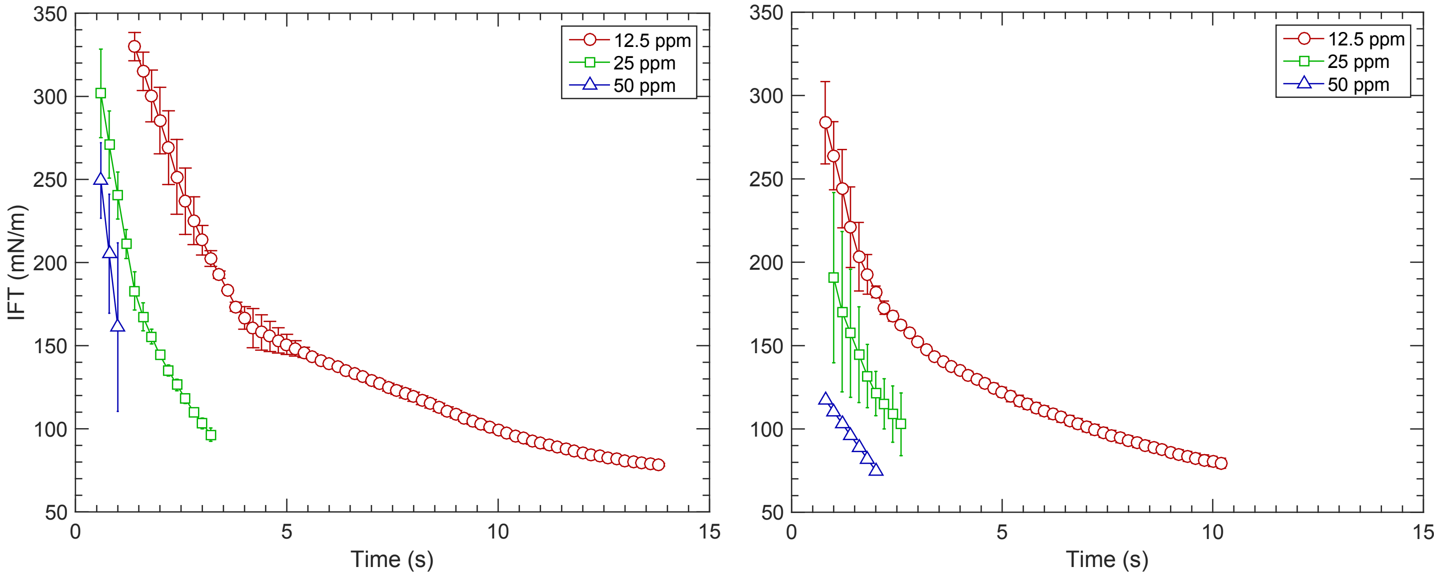

Figure C-3. IFT between a 3-μL oil droplet and (A) artificial freshwater or (B) artificial seawater. The artificial waters were dosed with various amount of 10 kDa to get a DOR of 12.5-100 ppm.

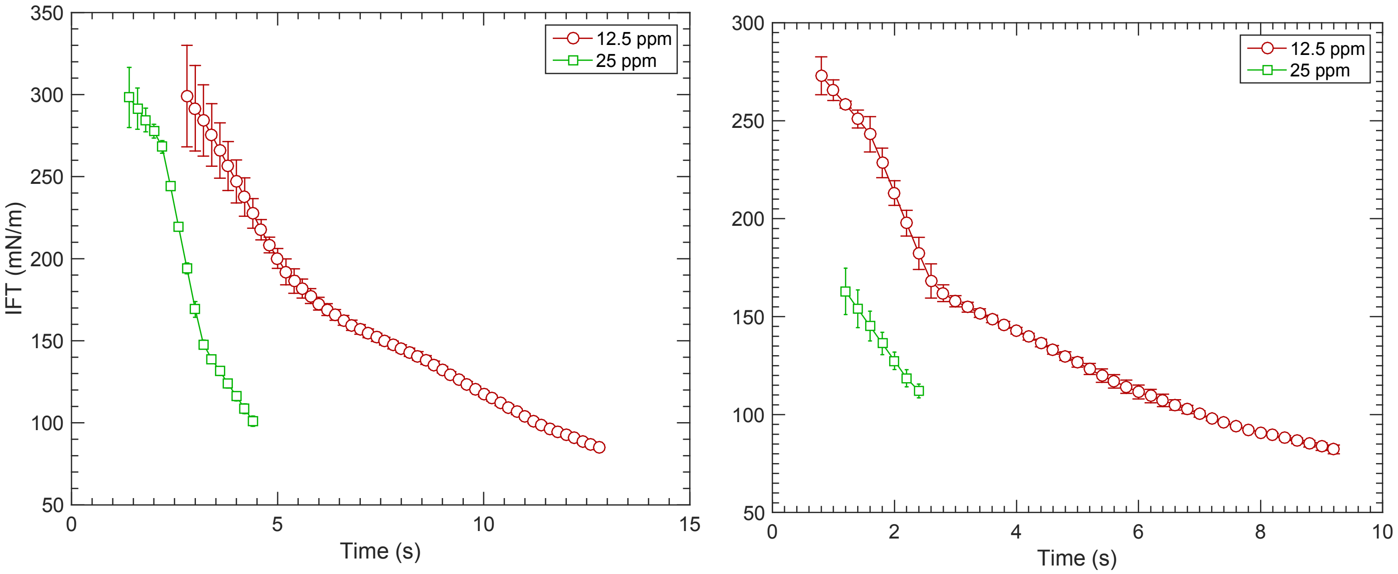

Figure C-4. IFT between a 3-μL oil droplet and (A) artificial freshwater or (B) artificial seawater. The artificial waters were dosed with various amount of 70 kDa to get a DOR of 12.5-100 ppm.

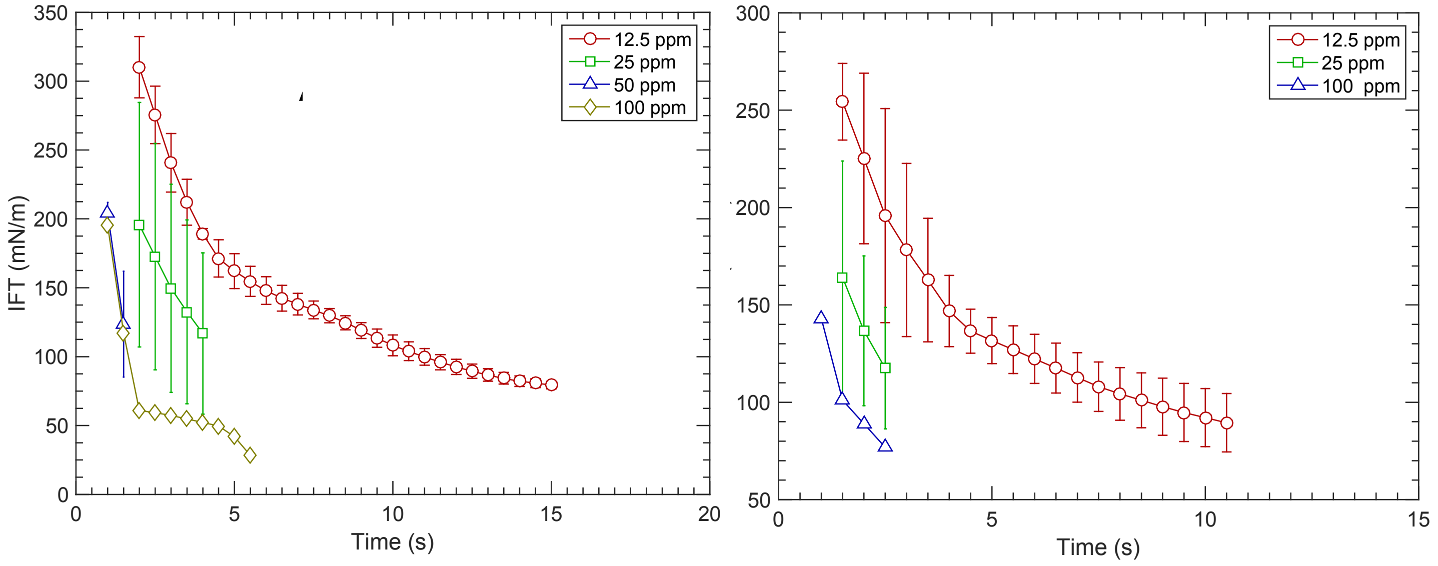

Figure C-5. IFT between a 3-μL oil droplet and (A) artificial freshwater or (B) artificial seawater (B). The artificial waters were dosed with various amount of 750 kDa to get a DOR of 12.5-100 ppm.

Appendix D – SOP's

Appendix D-1: Interfacial Tension, Pendant Drop, SOP

1. Turn on lamp on machine (back, right side)
2. Log onto computer and open up Drop Shape Analysis software
3. Click the fourth button from the left and the camera button
    a. Wave hand back and forth in front of camera on machine to insure that the camera is properly transmitting the image to the computer
4. Fill syringe with attach desired needle to syringe
5. Place syringe in the holder on the machine
    a. Loosen knob
    b. Press button on top of knob
    c. Insert in syringe, make sure aligned properly, may have to adjust height of syringe
    d. Tighten knob to secure syringe in place
6. Make sure the needle is in the center of the video screen
    a. Can adjust the height of the needle and syringe. Up/down = knob on the back of the syringe holder, left/right are the knobs on the front of the syringe holder
7. Fill cuvette with desired water and put on platform. Slowly raise platform to immerse the needle in the water
    a. First!! Make sure the needle is <u>CLEAN</u> if there is any contamination on the needle the oil will not form a drop
8. Adjust camera focus using the knob on the camera to make sure the needle in water is clear
    a. Open Focus Assistant to make sure it is reporting an acceptable number (will highlight green), if not play with focus, lighting (in DSA device control window), and location of cuvette until an acceptable number is achieved
9. Click on Profile → Calculate interfacial tension around Profile
10. Click on Options → Drop Type → Pendant Drop
11. Click on Options → Drop Type → Subtype → Bottom to Top
12. Move two lines on screen to be beneath the end of the needle (where the bottom of the oil droplet will be)
13. In the DSA device control window (if not open click the blue pipette button) select desired volume of oil to be dispensed. Then click the ^ button to push that volume of the syringe
    a. May have to repeat this step multiple times in the beginning to get out all the air in the syringe. Repeat until a full droplet forms.
14. Wait until the drop the forms releases itself from the needle (can gently flick needle to release the drop)
15. Click on Options → Tracker Man
16. In Tracker Man window click on Options and select the desired length of time for IFT measurement, Extract and Save Profile, and Save pictures (name them) if so desired
17. Click on the Table button
18. In Tracker Man Window click Start and then release an oil droplet (^ button in DSA device control window)

   a. A red or green line should outline the shape of the drop every x seconds (frequency input in Tracker Man window) and an IFT measurement should appear in the Table window. If this is not occurring make sure that the Focus Assistant is displaying an adequate number (highlighted in green). If not, adjust lighting and focus until an adequate number is displayed.

19. Once Tracker Man has completed the run (time is input in Tracker Man window) the pictures will be saved in the Drop Shape Analysis folder in documents. Copy and paste these in desired location. Highlight all the entries in the Table, Copy & Paste these in an Excel file.

Appendix D-2: Baffled Flask Test SOP [61]

Stock Standard Solutions

1. Weigh vial = x
2. Add 2-mL of oil → weigh vial + oil
3. Add dispersant for 1:25 DOR to the oil → weigh vial = y
4. Add 18-mL of DCM→weigh vial + oil + dispersant + DCM = z
5. Determine density of total vial (g/L) = ρ
6. Determine concentration of oil solution:

$$Conc\left(\frac{g}{L}\right) = \frac{y - x}{z - x/\rho}$$

Standard Solutions

1. For South Louisiana Crude specific volumes are:
    - 20-µL
    - 50-µL
    - 100-µL
    - 125-µL
    - 150-µL
2. Add specific volume of stock standard to 30-mL synthetic sea water in a 125-mL separatory funnel
3. Add 5-mL of DCM and shake for 15 seconds, let settle for 2 minutes
4. Drain 3-mL of DCM into a graduated cylinder
5. Repeat extraction two more times (drain to solvent water interface)
6. Adjust final solvent volume to 20-mL with DCM
7. Pour the 20-mL into a labeled screw top glass vial, tightly seal, and store in refrigerator until

BFT

1. Add 120-mL of artificial seawater into the modified baffled flask
2. Add 100-µL oil to the baffled flask
3. Add 50-µL of dispersant onto the center of the oil slick to achieve a 1:25 DOR
4. Mix flask on orbital shaker for 10 minutes at 200 rpm
5. Let flask settle for 10 minutes
6. Using the stop cock discard the first 2-mL of the sample
7. Using the stop cock gather 30-mL of sample into a graduated cylinder
8. Put the 30-mL into 125-mL separatory funnel

9. Add 5-mL DCM to separatory funnel and shake by hand for 5 minutes/25 times.
10. Release pressure from separatory funnel into fume hood
11. Let separatory funnel settle for 2 minutes
12. Drain 3-mL of DCM layer into graduated cylinder
13. Add 5-mL DCM to the separatory, mix, and drain two more times (combine all extracts together).
14. Adjust final volume of extracts to 20-mL with DCM

Spectrophotometer

1. Warm up spectrophotometer for 30 minutes and bring up all standards and samples to room temperature.
2. The blank is seawater (no oil or dispersant) that has undergone a BFT run.
3. After blanking, measure six calibration standards at 340, 370, and 400 nm with quartz cuvettes (covered) and record absorbance.
4. Measure samples at 340, 370, and 400 nm with quartz cuvettes (covered) and record absorbance
5. It is easiest to blank at 340 nm, run the six calibrations, and then the samples. Then do the same series with 370 and 400 nm.
6. Record all absorbences to make calibration curves to calculate dispersion effectiveness.

REFERENCES

[1] National Research Council, “Oil in the sea,” Washington, D.C., 1985.

[2] U.S. Coast Guard, “Update of inputs of petroleum hydrocarbons into the oceans due to marine transportation activities,” *Natl. Res. Counc.*, vol. National A, no. Washington, D.C., 1990.

[3] J. A. Anderson Lively and J. McKenzie, “Toxicity of the dispersant Corexit 9500 to early life stages of blue crab , *Callinectes sapidus*,” *Bull Environ. Contam. Toxicol.*, vol. 93, pp. 649–653, 2014.

[4] G. Goodbody-gringley, D. L. Wetzel, D. Gillon, E. Pulster, A. Miller, and K. B. Ritchie, “Toxicity of Deepwater Horizon source oil and the chemical dispersant , Corexit H 9500 , to coral larvae,” *PLoS One*, vol. 8, no. 1, pp. 1–10, 2013.

[5] R. R. Lessard and G. Demarco, “The significance of oil spill dispersants,” *Spill Sci. Technol. Bull.*, vol. 6, no. 1, pp. 59–68, 2000.

[6] H. Chapman, K. Purnell, R. J. Law, and M. F. Kirby, “The use of chemical dispersants to combat oil spills at sea : A review of practice and research needs in Europe,” *Mar. Pollut. Bull.*, vol. 54, pp. 827–838, 2007.

[7] International Tanker Owners Pollution Federation, “Oil tanker spill information pack,” *London: International Tanker Owners Pollution Federation*, 2015. .

[8] The American Petroleum Institute, “Crude oil category,” *US EPA-Category Assess. Doc.*, vol. 201–16852B, pp. 1–58, 2011.

[9] P. F. Kingston, “Long-term environmental impact of oil spills,” *Spill Sci. Technol. Bull.*, vol. 7, no. 02, pp. 53–61, 2002.

[10] R. C. Prince, R. R. Lessard, and J. R. Clark, “Bioremediation of marine oil spills,” *Oil Gas Sci. Technol.*, vol. 58, no. 4, pp. 463–468, 2003.

[11] R. Pallardy, “Deepwater Horizon oil spill of 2010; oil spill, Gulf of Mexico,” *Encyclopedia Britannica Online*, 2016. .

[12] C. Kilduff and J. Lopez, “Dispersants: the lesser of two evils or a cure worse than the disease," *Ocean Coast. L.J.*, vol. 375, pp. 375–381, 2011.

[13] L. C. Smith, L. M. Smith, and P. A. Ashcroft, “Analysis of environmental and economic damages from British Petroleum’s Deepwater Horizon oil spill,” *Albany Law Rev.*, vol. 74, no. 1, pp. 563–585, 2011.

[14] U.S. Department of Interior, “Interior fact sheet - BP Deepwater Horizon response,” *U.S. Department of Interior*, 2010. [Online]. Available: http://www.doi.gov/deepwaterhorizon/Interior-Fact-Sheet-BP-Deepwater-Horizon-Response.cfm. [Accessed: 11-Jun-2016].

[15] J. K. Drapkin, “10 animals most at risk from gulf oil spill,” *CBS News*, 2010. .

[16] U.S. Fish and Wildlife Service, “FWS Deepwater Horizon oil spill response,” *U.S. Fish and Wildlife Service*, 2010. [Online]. Available: http://www.fws.gov/home/dhoilspill/index.html. [Accessed: 11-Jun-2016].

[17] United States Environmental Protection Agency, “EPA response to BP spill in the Gulf of Mexico,” *EPA*, 2016. [Online]. Available: https://archive.epa.gov/bpspill/web/html/dispersants-qanda.html. [Accessed: 10-Jun-2016].

[18] B. C. Harris, J. S. Bonner, T. J. McDonald, C. B. Fuller, and C. A. Page, “Bioavailability of chemically-dispersed crude oil,” in *Proceedings of the Twenty-fifth Arctic and Marine Oil Spill Conference, Environment Canada, Ottawa*, 2002, pp. 895–905.

[19] R. C. Prince, “Petroleum and other hydrocarbons, biodegradation of,” *Encycl. Environ. Microbiol.*, 2003.

[20] C. E. Zobell, “Microbial modification of crude oil in the sea,” in *Proceedings of API-FWPCA Meeting*, 1969, p. 317ff.

[21] J. G. Leahy and R. R. Colwell, “Microbial degradation of hydrocarbons in the environment,” *Microb. Rev.*, vol. 54, no. 3, pp. 305–315, 1990.

[22] Z. Cui, Q. Lai, C. Dong, and Z. Shao, “Biodiversity of polycyclic aromatic hydrocarbon-degrading bacteria from deep sea sediments of the Middle Atlantic Ridge,” *Environ. Microbiol.*, vol. 10, pp. 2138–2149, 2008.

[23] T. Hazen, “Oil-eating microbes,” *Lawrence Berkeley National Lab*, 2010. [Online]. Available: http://www.cleveland.com/business/index.ssf/2010/06/oil-eating_microbes_seen_as_po.html. [Accessed: 10-Jun-2016].

[24] P. Campo, A. D. Venosa, and M. T. Suidan, “Biodegradability of Corexit 9500 and dispersed south Louisiana crude oil at 5 and 25 ° C,” *Environ. Sci. Technol.*, vol. 47, no. 4, pp. 1960–1967, 2013.

[25] R. Proctor, A. J. Elliott, and R. A. Flather, “Forecast and hindcast simulations of the *Braer* oil spill,” *Marine Pollution Bulletin*, vol. 28, no. 4, pp. 219–229, 1994.

[26] R. C. Prince, “Oil spill dispersants: boon or bane?,” *Environ. Sci. Technol.*, vol. 49, no. 11, pp. 6376–6384, 2015.

[27] M. Bollmann, T. Bosch, F. Colijn, R. Ebinghaus, R. Froese, K. Güssow, S. Khalilian, S. Krastel, A. Körtzinger, M. Langenbuch, M. Latif, B. Matthiessen, S. P. Frank Melzner, Andreas Oschlies, J. R. Alexander Proelß, Martin Quaas, P. R. Till Requate, Thorsten Reusch, H. S. Jörn O. Schmidt, Kerstin Schrottke, U. S. Ursula Siebert, Rüdiger Soltwedel, T. T. Karl Stattegger, Horst Sterr, Renate Sturm, C. van B. Athanasios Vafeidis, M. V. Justus van Beusekom, Rüdiger Voss, and F. W. Martin Wahl, Klaus Wallmann, *World Ocean Review-Living with the oceans*. maribus, 2010.

[28] A. George-ares, R. R. Lessard, G. P. Canevari, and K. W. Becker, "Modification of the dispersant Corexit ® 9500 for use in freshwater," *Int. Oil Spill Conf.*, vol. 2001, pp. 1209–1211, 2001.

[29] M. Fingas, "Dispersants , salinity and Prince William Sound," *Prince William Sound Reg. Citizens' Advis. Counc.*, pp. 1–48, 2004.

[30] R. Rico-martínez, T. W. Snell, and T. L. Shearer, "Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A Ò to the *Brachionus plicatilis* species complex (Rotifera)," *Environ. Pollut.*, vol. 173, pp. 5–10, 2013.

[31] S. E. Anderson, J. Franko, E. Lukomska, B. J. Meade, S. E. Anderson, J. Franko, E. Lukomska, and B. J. Meade, "Potential immunotoxicological health effects following exposure to COREXIT 9500A during cleanup of the Deepwater Horizon oil spill," vol. 7394, no. June 2016, 2011.

[32] K. J. Wooten, B. E. Finch, and P. N. Smith, "Embryotoxicity of Corexit 9500 in mallard ducks (*Anas platyrhynchos*)," *Ecotoxicology*, vol. 21, no. 3, pp. 662–666, 2012.

[33] G. Pi, L. Mao, M. Bao, Y. Li, H. Gong, and J. Zhang, "Preparation of oil-in-seawater emulsions based on environmentally benign nanoparticles and biosurfactant for oil spill remediation," *ACS Sustain. Chem. Eng.*, vol. 3, no. 11, pp. 2686–2693, 2015.

[34] Nalco, "COREXIT Ingredients," *Nalco Environmental Solutions LLC.*, 2014. .

[35] European Maritime Safety Agency, "Manual on the applicability of oil spill dispersants - version 2," p. 10, 2009.

[36] R. Duncan and L. Izzo, "Dendrimer biocompatibility and toxicity," *Adv. Drug Deliv. Rev.*, vol. 57, pp. 2215–2237, 2005.

[37] H. Klinkmann, H. Wolf, and E. Schmitt, "Definition of biocompatibility," *Risk Profilies Clin. Nephrol.*, vol. 37, pp. 70–77, 1984.

[38] J. R. Bragg, R. C. Prince, E. J. Harner, and R. M. Atlas, "Effectiveness of bioremediation for the *Exxon Valdez* oil spill," *Nature*, vol. 368, pp. 413–418, 1994.

[39] R. C. Prince and J. R. Bragg, "Shoreline bioremediation following the *Exxon Valdez* oil spill in Alaska shoreline bioremediation following the *Exxon Valdez* oil spill in Alaska," *Bioremediat. J.*, vol. 1, no. 2, pp. 97–104, 1997.

[40] R. Atlas and J. Bragg, “Bioremediation of marine oil spills : when and when not – the Exxon Valdez experience,” *Microb. Biotechnol.*, vol. 2, no. 2, pp. 213–221, 2009.

[41] A. D. Venosa, J. R. Haines, and D. . Allen, “Efficacy of commercial inocula in enhancing biodegradation of weathered crude oil contaminating a Prince William Sound beach,” *J. Ind. Microbiol.*, vol. 10, pp. 1–11, 1992.

[42] G. Pi, Y. Li, M. Bao, L. Mao, H. Gong, and Z. Wang, “Novel and environmentally friendly oil spill dispersant based on the synergy of biopolymer Xanthan Gum and silica nanoparticles,” *ACS Sustain. Chem. Eng.*, vol. 4, no. 6, pp. 3095–3102, 2016.

[43] S. M. Grayson, “Development of cost-efficient and concentration-independent dispersants for improved oil spill remediation,” *Gulf of Mexico Research Initiative*, 2016.

[44] E. Muhammad, A. M. Alb, A. Kosakowska, Karolina, and S. M. Grayson, “Modular amphiphilic copolymer-grafted nanoparticles: ‘nanoparticle micelle’ behavior enhanced utility as dispersants,” *Polym. Chem.*, vol. 6, pp. 7749–7757, 2015.

[45] N. K. Geitner, R. R. Powell, T. Bruce, and A. Ladner, “Effects of dendrimer oil dispersants on *Dictyostelium discoideum*,” *RSC Adv.*, vol. 3, pp. 25930–25936, 2013.

[46] X. Shi, S. Wang, H. Sun, and J. R. Baker, “Improved biocompatibility of surface functionalized dendrimer- entrapped gold nanoparticles,” *R. Soc. Chem.*, vol. 3, pp. 71–74, 2007.

[47] A. Quintana, E. Raczka, L. Piehler, I. Lee, A. Myc, I. Majoros, A. K. Patri, T. Thomas, J. Mule, and J. R. Baker, “Design and function of a dendrimer-based therapeutic nanodevice targeted to tumor cells through the folate receptor,” *Pharm. Res.*, vol. 19, no. 9, pp. 1310–1316, 2002.

[48] T. P. Thomas and C. B. Mehta, “Poly(amidoamine) dendrimer-based multifunctional engineered nanodevice for cancer therapy,” *J. Med. Chem.*, vol. 48, no. 19, pp. 5892–5899, 2005.

[49] Y. Huang, D. Wang, X. Zhu, D. Yan, and R. Chen, “Synthesis and therapeutic applications of biocompatible or biodegradable hyperbranched,” *Polym. Chem.*, vol. 6, pp. 2794–2812, 2015.

[50] S. Peleshanko and V. V. Tsukruk, “The architectures and surface behavior of highly branched molecules,” *Prog. Polym. Sci.*, vol. 33, pp. 523–580, 2008.

[51] Y. Tu, “Dendritic polymers as biocompatible oil spill dispersants: effectiveness and mechanisms with crude oil,” *M.S. Thesis from Clemson University*, 2014.

[52] N. K. Geitner, P. Bhattacharya, M. Steele, R. Chen, D. A. Ladner, and P. C. Ke, “Understanding dendritic polymer-hydrocarbon interactions for oil dispersion,” *RSC Adv.*, vol. 2, pp. 9371–9375, 2012.

[53] T. J. Norberg-King, L. W. Ausley, D. T. Burton, W. L. Goodfellow, J. L. Miller, and W. T. Waller, *Toxicity Reduction and Toxicity Identification Evaluations for effluents, ambient waters, and other aqueous media*. Society of Environmental Toxicology and Chemistry, 2005.

[54] M. Salehi, Y. Tu, A. Boettcher, N. Geitner, D. Ladner, K. Carpenter, S. Powers, and A. J. Whelton, "Influence of hyperbranched polymers and Corexit 9500 on crude oil dispersion and *Daphnia magna*," *Pending*, 2016.

[55] Y.-B. Lim, S.-O. Han, H.-U. Kong, Y. Lee, J.-S. Park, B. Jeong, and S. W. Kim, "Biodegradable polyester, poly[α-(4-aminobutyl)-l-glycolic acid], as a non-toxic gene carrier," *Pharm. Res.*, vol. 17, no. 7, pp. 811–816, 2000.

[56] United States Envrionmental Protection Agency, *Procedures for Conducting Daphnia magna Toxicity Bioassays*. 1987, pp. EPA/600/8–87/011.

[57] G. Anderson, "Seawater composition," *Marine Science*, 2008.

[58] R. Reul, J. Nguyen, and T. Kissel, "Biomaterials Amine-modified hyperbranched polyesters as non-toxic , biodegradable gene delivery systems," *Biomaterials*, vol. 30, no. 29, pp. 5815–5824, 2009.

[59] N. K. Geitner, B. Wang, R. E. Andorfer, D. A. Ladner, P. C. Ke, and F. Ding, "Structure − function relationship of PAMAM dendrimers as robust oil dispersants," *Environ. Sci. Technol.*, vol. 48, no. 21, pp. 12868–75, 2014.

[60] A. D. Venosa, D. W. King, and G. A. Sorial, "The Baffled Flask Test for dispersant effectiveness : A round robin evaluation of reproducibility and repeatability," *Spill Sci. Technol. Bull.*, vol. 7, no. 5–6, pp. 299–308, 2002.

[61] A. D. Venosa, E. Holder, and N. Star, "Laboratory-scale testing of dispersant effectiveness of 20 oils using the Baffled Flask Test," *U.S. Environmental Protection Agency*, 2011. [Online]. Available: http://www.bsee.gov/Technology-and-Research/Oil-Spill-Response-Research/Reports/600-699/666AA/ [Accessed: 01-Jun-2016].

[62] "The Baffled Flask Test standard operating procedure for determining effectiveness of dipsersants, version 5.0, October 15, 2001,"

[63] L. Ravina, *Everything you want to know about Coagulation & Flocculation ....* Zeta Meter, 1993.