ULTRAVIOLET FLUORESCENCE SPECTROSCOPY (UVFS):
A NEW MEANS OF DETERMINING THE EFFECT OF
CHEMICAL DISPERGANTS ON OIL SPILLS

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ABSTRACT
Crude oils dispersed in seawater produce distinct emission spectra when exposed to ultraviolet (UV) light. The spectra can be used to estimate how effectively oil is dispersed by chemical methods. Oil dispersants (such as Corexit 9500) have a pronounced effect on water-based UV spectra, strongly enhancing emission at 445 nm. This enhancement of fluorescence over the 455 nm bandwidth is the result of dispersant breaking up higher molecular weight (> 3 ring) polycyclic aromatic hydrocarbons (PAHs) into stable suspensions of small droplets. Ultraviolet fluorescence spectroscopy (UVFS) has been tested as a rapid analytical tool in the laboratory and in a wave tank designed to investigate the response of crude oils to dispersants and a range of energy dissipation rates. The results obtained with UVFS are consistent with standard chemical analyses, confirming that the method can be employed as a rapid, quantitative measure of dispersed oil concentration. Given that higher molecular weight PAHs are associated with many of the persistent toxic effects of crude oils on marine organisms, UVFS may also prove to be a useful tool for tracking these fractions during dispersed oil toxicity assays.

INTRODUCTION
Oil can be released into the environment from a number of sources (National Research Council, 1985) and cause serious environmental damage if not remediated. In order to implement a practical remediation strategy, the oil and corresponding by-products of remediation need to be identified and tracked. Methods such as gas chromatography-flame ionization detection (GC-FID), gas chromatography-mass spectroscopy (GC-MS), high-performance liquid chromatography (HPLC) and ultraviolet absorbance (UVA) are all sensitive tracer techniques, but require the extraction and concentration of samples prior to analysis. This means that a great deal of time is spent on the quantitative measurement and interpretation of the data (Muroski et al., 1996; Booksh et al., 1996; Jiji et al., 1999; Venosa et al., 2002; Nahorniak and Booksh, 2003; Ryder et al., 2003; Ryder, 2004; Christensen et al., 2005). Any method that allows a more direct and rapid interpretive analysis of aquatic samples would be a useful addition to this battery of techniques.

Ultra-violet fluorescence spectroscopy (UVFS) has already been shown to be a rapid and sensitive means of obtaining information on the presence and relative concentration of many organic compounds in seawater (Østgaard and Jensen, 1983; Coble et al., 1996; Muroski et al., 1996; Booksh et al., 1996; Jiji et al., 1999; Nahorniak and Booksh, 2003; Christensen et al., 2005). As Patra and Mishra (2002) have pointed out, it is a particularly useful tool because it does not require the extraction and concentration procedures that are typical of other spectroscopic techniques like UV-visible, infrared (IR), Raman scattering and nuclear magnetic resonance (NMR). When applied to water samples (Coble, 1996, with references), UVFS has provided detailed information to identify natural dissolved organic matter (DOM). Fluorescence spectroscopy has also been utilized to qualitatively estimate crude oil concentrations in natural waters (Keizer and Gordon, 1973; Østgaard and Jensen, 1983). In addition, Kepkay et al. (2002) have used UVFS as a means of distinguishing between oil dispersed in seawater and the oil incorporated into oil-mineral aggregates (OMAs).

The fact that many organic compounds fluoresce at specific excitation and emission wavelengths is the basis for identifying many of the components of DOM and crude oil in seawater. For example, humic material fluoresces over emission wavelengths of 320-350 nm when subject to excitation at 230-260 nm, and proteins fluoresce over emission wavelengths of 300-350 nm with excitation at 220 and 275 nm (Coble, 1996, with references). In addition, when subject to excitation at 245-280 nm, polycyclic aromatic hydrocarbons (PAH) fluoresce over wavelengths of 310 to > 400 nm, depending on the number of aromatic rings in the structure (Wakeman, 1977; Von der Dick and Kalkreuth, 1985; Biddleman et al., 1990; Patra and Mishra, 2002; Bugden et al., 2007). However, the two-dimensional (2D) emission spectra of crude oils extracted into a variety of solvents (and subject to excitation at a single wavelength) generally resolve into a broad, featureless peak that extends over the full bandwidth of 310 to > 400 nm (Biddleman et al., 1990; Nahorniak and Booksh, 2003; Christensen et al., 2005).

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Laboratory Experiments. The test oil was MESA crude (Petro-Canada, Montreal, PQ) with an API (American Petroleum Institute) gravity of 29.7° that had been artificially weathered by aeration to 86.2% of original weight. The dispersant applied to suspensions of the oil in seawater was Corexit 9500® (Nalco Energy Service, L.P. Sugar Land TX).

In one set of experiments, 5 mL of oil were dispersed into 50 mL of 1-μm filtered seawater (at a nominal concentration of 100 ppm of oil) in a 90 mL specimen container (Fisher Scientific) using a Rainin LTS pipettor. In order to ensure that no oil remained in the pipet tip, the tip was rinsed three to five times with seawater, and each residue injected into the specimen cup. The oil/water sample was then placed on a reciprocal shaker (Eberbach Corp., Ann Arbor, Michigan) at approximately 280 oscillations per minute and shaken for 30 minutes to physically disperse the oil.

To assess the influence of dispersant on the oil, a second set of experiments was performed, where 5 mL of oil was measured into a 20 mL borosilicate scintillation vial (FisherScientific) and 250 μL of dispersant (Corexit 9500®) was added to produce a 1:20 dispersant to oil ratio (DOR). This ratio was chosen based on the observation that one part dispersant will disperse about 20–30 parts of oil (Lassard and DeMarco, 2000), and falls within the range of 1:10 and 1:30 used by other researchers (Page et al., 2000; Yamada et al., 2003; Couillard et al., 2005). The oil/ dispersant mixture was vigorously shaken by hand for approximately 60 seconds prior to dispersion in 50 mL of seawater as described above. Duplicate runs for each oil and each oil-dispersant combination were also performed.

At the end of each 30-minute dispersion experiment, 3 mL of the oil-water or oil-dispersant-water mix were transferred into a 4.5 mL ultraviolet-grade methacrylate disposable cuvette (10 mm light path - VWR International Inc., Mississauga, ON) using a Gilson P5000 Pipetman and Gilson D5000 5 mL tips. Ultraviolet (UV) fluorescence spectra were obtained with a QM-1 scanning spectrofluorometer running FeliX software (PTI Inc., Birmingham, AL) and with the excitation wavelength fixed at 280 nm. Scans of 1-μm filtered seawater and seawater plus Corexit 9500 were obtained in the same manner as the oil and oil plus dispersant samples for comparison to spectra obtained with progressively-increasing concentrations of oil added in suspension.

Wave Tank Experiments. The wave tank facility at Bedford Institute of Oceanography (Dartmouth, Canada) has been described in detail by Li et al. (2007a,b). The facility is equipped with a wave generator that can produce recurrent breaking waves at the same location. A dispersive focusing technique was utilized, where a wave of one frequency was superimposed on a wave of another frequency, causing the wave to break under different inertial forces at a predefined location. As described by Li et al. (2007a), the calibration of non-breaking wave and breaking-wave energy was expressed in terms of the energy dissipation rate (ε).

The procedure followed to determine the effect of dispersant and mixing energy on the dispersion of MESA crude was identical to the two-factor, mixed-level, experimental design of Li et al. (2007b). Following the addition of oil or oil then dispersant to the surface of breaking and non-breaking waves, samples were taken over a range of depths and locations in the tank to be analyzed for oil concentration. Two analytical techniques were applied to the samples: First, the 2D ultraviolet fluorescence spectroscopy (UVFS) technique was utilized (following the protocol developed for laboratory experiments) and second, the standardized UV absorption (UVA) technique, (based on the protocol of Venosa et al., 2002; and Chandrasekar et al., 2005) was used. This allowed oil concentrations determined by UVFS to be compared to concentrations measured by the more widespread and standard technique of UVA in samples from a number of wave tank experiments.

RESULTS AND DISCUSSION

Laboratory Experiments. Oils dispersed in seawater produce distinct emission spectra when exposed to ultraviolet light (Bugden et al., 2007 with references). For example, MESA crude dispersed into seawater in the laboratory and exposed to UV radiation at an excitation wavelength of 280 nm produces an emission spectrum (Fig 1) that is typical of many low-viscosity oils: The high intensity observed at an emission wavelength of 340 nm is probably associated with 1- and 2-ring PAHs (Wakeman, 1977). The broader, less-intense peak spanning emission wavelengths of 400 to 500 nm (centered at 445 nm) is associated with higher molecular weight (> 3-ring) PAHs (Wakeman, 1977; Von der Dick and Kalkreuth, 1986; Smith and Sinski, 1999; Patra and Mishra, 2002).

FIG. 1: UV EMISSION SPECTRA OF MESA CRUDE DISPERSED IN SEAWATER WITH (RED) AND WITHOUT (BLUE) THE ADDITION OF COREXIT 9500 AS A DISPERSANT.

The effect of Corexit 9500 on these water-based ultra violet fluorescence (UVF) spectra is pronounced, with emission at 445 nm enhanced (Fig. 1) to the level where intensity at 445 nm is almost equal to the intensity at 340 nm. Yamada et al. (2003) and Couillard et al. (2005) have already shown that Corexit increases the aqueous concentrations of less water-soluble, high molecular weight PAHs (with >3 rings) compared to low-molecular weight PAHs (with one or two rings). The increase at 445 nm can be explained by the action of dispersant on the higher-molecular weight PAH fraction to increase its aqueous concentration by generating
a larger number of small droplets dispersed into the water column (Lessard and Demarco, 2000; Page et al., 2000). Bugden et al. (2007) have also found that the enhancement of the 445 nm emission peak by the action of dispersant is typical of oils with a wide (14,470-fold) range of viscosities, and that the action of dispersant can be expressed in terms of a ratio of emission at 340 nm divided by emission at 445 nm.

The advantage inherent in the application of emission ratios is that dispersion could be tracked without having to measure oil concentration to calibrate the UVF spectra. However, it is also possible to take the changes associated with the addition of Corexit (such as those apparent in Fig. 1), and use them to calibrate the spectra and quantify the effect of increasing oil or oil plus dispersant. The spectral changes associated with increasing concentrations of MESA crude are plotted in Fig. 2. These changes are not only characterized by an overall increase in spectral intensity linked to the increase in oil concentration (Fig. 2A), they are also associated with the "Corexit effect" observed by Bugden et al. (2007), where the increase in emission intensity at 445 nm is far more pronounced than the increase at 340 nm (Fig. 2B).

When these spectral changes are quantified in terms of oil concentration (Fig. 3), it is clear that linear standard curves of oil concentration can be generated from the spectra. It is also clear that two sets of standard curves need to be generated, one for oil only and one for oil plus dispersant in the seawater.

The fact that the UVFS and UVA data were comparable at either 445 nm or over the whole spectrum of intensities suggests that the fate of higher molecular weight (> 3-ring) PAH fractions will provide a good idea of the fate of oil as a whole during the
dispersion process. This finding is particularly important, given that higher molecular weight PAHs are associated with many of the persistent toxic effects of crude oils on marine organisms (Anderson et al. 1974). As a result, UVFS appears to be a particularly useful tool for quantitative measurements of the kinetics of oil dispersion and reaccumulation. When combined with the fact that UVFS (unlike UVA and other techniques) requires no extraction and concentration of oil from seawater (Patra and Mishra, 2002), fluorescence spectroscopy shows promise as a rapid analytical tool for the monitoring of oil slick dispersion.

**SUMMARY**

Dispersant effectiveness cannot be defined on the basis of any one approach. An extensive arsenal of analytical tools is required to arrive at any practical definition of effectiveness. There are a number of reasons why ultraviolet fluorescence spectroscopy (UVFS) is an important addition to this arsenal:

1. UVFS is a rapid and sensitive means of obtaining information on the presence and concentration of oil dispersed in seawater because it does not require the extraction and concentration procedures that are typical of other standard techniques, such as ultraviolet absorption (UVA).
2. The effect of oil dispersion on UVFS spectra can be expressed in terms of an emission ratio, so that dispersion can be tracked without having to measure oil concentration. The spectral changes associated with the application of dispersant (such as those apparent in Fig. 1) can also be calibrated to quantify increasing oil or oil plus dispersant.
3. The fact that UVFS and UVA data are comparable at an emission intensity of 445 nm or over the whole spectrum of intensities (from 300 - 500 nm) indicates that the fate of higher molecular weight (> 3-ring) PAH fractions - the more "dispersible" fraction of an oil slick - will provide a good idea of the fate of the oil as a whole during the dispersion process.
4. Given that higher molecular weight PAHs may be associated with many of the persistent (or chronic) toxic effects of crude oils on marine organisms, the ability of UVFS to track "dispersible" fractions would make it a particularly useful tool in studies of the long-term toxic effects of dispersed oil.

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**REFERENCES**


