

Qualification of Oil-spill Treatment Products

Protocol for efficacy testing

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1 Introduction

1.1 Background

Oil spills from offshore oil and gas production as well as ship traffic can have disastrous effects on the marine environment (Pezeshki et al., 2000, Helle et al., 2016). Hazardous short-term effects include toxic effects from the oil itself, reduction of light in the water column and therefore reduction of primary production from algae, as well as choking of local fauna such as marine mammals (Albers, 2003, NRC 2003, Penela-Arenaz et al., 2009).

The use of dispersants can help in the mitigation of these acute effects by dispersing the oil through the water column, thereby breaking oil layers that have formed on the surface of the water close to the spill side (Brakstad et al., 2015). This effect can potentially increase the biodegradation rate of the spilled oil, reduces the risk of animals choking on the oil or starving due to low primary production (Hazen et al., 2010, Silva et al., 2015).

However, dispersants can be hazardous to the environment if they themselves have harmful ecotoxicological properties (Rahsepar et al., 2016). Furthermore, dispersants do not reduce the amount of oil entering the environment and can add to the toxic effects of the spill underwater since dispersants and dispersed oil under the surface can still be hazardous for marine life (Rahsepar et al., 2016).

In the UK, the Marine and Coastal Access Act 2009 (Great Britain- Parliament, 2009) requires any substance to be licensed before it can be discharged into UK waters. Therefore, no dispersant can be used in the UK unless it has been approved by the appropriate UK authority, the Secretary of State for the Department for Environment, Food and Rural Affairs (Defra), the Scottish Executive, the Welsh Assembly Government, or the Department of the Environment for Northern Ireland (DoE(NI)). To be approved, a dispersant has to meet criteria regarding its efficacy (effectiveness) as well as toxicity.

The presented method for testing dispersant efficiency was adopted from the 'Baffled Flask Test' (BFT) method used by the US EPA for the determination of the efficiency of oil spill dispersants (Venosa et al., 2002, Holder et al., 2015).

1.2 Percentage of oil dispersed

The percentage of oil dispersed (%OD) by an oil spill dispersant is defined as the percentage of the test oil by weight which has been dispersed into the water phase under the conditions of the test.

1.3 Types of Dispersant

This specification relates to three types of oil spill dispersant. These are:

Type 1: Conventional Hydrocarbon-base - for use primarily undiluted on beaches, but may also be used undiluted at sea from Warren Springs Laboratory (WSL) spray sets using breaker boards or other suitable means of application and agitation.

Type 2: Water-dilutable concentrates - for use at sea after dilution 1:10 with seawater, and sprayed from WSL spray sets using breaker boards or other suitable means of application and agitation.

Type 3: Concentrate - for use undiluted from aircraft, ships or on beaches, using appropriate spray gear.

2 Dispersant efficacy testing

2.1 Equipment /Apparatus:

Modified Trypsinizing Flask: 150 mL glass Trypsinizing flasks with a glass stopcock near the bottom.

Platform shaker: A platform shaker with a variable speed control unit (40-400 rpm) and an orbital diameter of approximately 0.75 inches (2 cm) to create rotational mixing in the test flask liquids.

Micropipettor: An Eppendorf Multipette plus, Sartorius eLINE dispenser or similar repeater pipette using positive displacement tips capable of dispensing 16 μ L of dispersant and 400 μ L of oil.

Far UV quartz (170-2700nm) cuvettes/ screw-cap cuvettes with 10mm path length

Glassware: Glassware consisting of 25 and 100 mL volumetric flasks, 250 mL separating funnels with Teflon stopcocks, disposable glass Pasteur pipettes and other standard lab glassware.

Syringes: 50, 100 and 1000 μ L gas-tight syringes.

Spectrophotometer: A spectrophotometer capable of measuring absorbance from 340 to 400 nm and suitable for cuvettes of 10mm path length.

Analytical balances: Balances calibrated as per standard laboratory procedures.

2.2 Ingredients/Reagents/Media

Test Oils: The oil used in the dispersant efficacy test is Kuwait Crude Oil (batch 04-08-11). Kuwait Crude oil is a medium crude oil that is used as the standard reference oil for the toxicity testing of dispersants (Kirby et al. 1996). The use of Kuwait crude oil, therefore allows for harmonisation across the different steps in the dispersant licensing process. The characteristics of Kuwait crude oil were:

- Density at 25°C: 0.8768 kg/l
- Dynamic viscosity at 10°C: 279.3 cSt
- Pour point (IP 15/67): <5°C

Seawater: natural, collected from Lowestoft Bay, settled and then filtered at 20 microns prior to use.

Methylene Chloride: Dichloromethane (DCM), pesticide grade, for extraction of all sample water and oil-standard water samples.

Dispersants: Test substance for qualification.

3 Test Procedure

During the preparation, handling and spiking of standards disposable nitrile gloves, fastened lab coat and safety glasses are required to be worn and all work carried out in a local exhaust ventilation (LEV).

3.1 Sample Handling, Collection, and Preservation

All glassware is rinsed with DCM, soaked in warm water with detergent and individually washed with bristled brushes prior to use. The glassware is then rinsed with tap water, followed by two de-ionized water rinses, dried at 110 °C and rinsed with fresh DCM.

Samples of oil in DCM should be kept in glass stoppered mixing cylinders until being brought up to volume. After being brought to volume they should be stored under refrigeration at 5±3 °C. Stock standard oil and dispersant should be kept at room temperature.

All standards are allowed to equilibrate to room temperature before use.

3.2 Calibration

The theoretical concentration of the stock standard solution is calculated from the mass of oil in mg divided by the total mass oil/dispersant/DCM solution divided by its density in g/ml (equation 1). The density of the oil/dispersant/DCM solution is measured gravimetrically using a density bottle or a 1 mL gas tight syringe. The theoretical oil concentration in the stock solution is calculated according to the following equation:

$$\text{theoretical concentration, } \frac{\text{mg}}{\text{mL}} = \frac{\text{mass of oil, g} * 1000}{\text{total mass, g} / \rho_{\text{solution, g/mL}}} \quad (1)$$

The stock standard solutions are prepared from the reference oil and the specific dispersant that is to be assessed. The stock solution of dispersant-oil mixture in DCM is prepared using 2 mL of oil, then adding 3 mL of the dispersant followed by 20 mL of DCM.

Five-point Calibration Curve:

For each calibration point, specific volumes of its stock standard solution (given in Table 1) are added to 30 mL of artificial seawater in a 125 mL separating funnel and extracted with triplicate 4 mL volumes of DCM. Each DCM addition is followed with 15 seconds of vigorous shaking, carefully releasing the initial pressure inside the separating funnel by partially removing the glass stopper inside a fume hood after the first few shakes.

The shaking is followed by a 2-minute stationary period for phase separation for each extraction. Extracts are drained into a 25 mL graduated mixing cylinder, releasing any entrained bubbles of DCM from the water layer by sideways shaking of the funnel.

The final volume of each of the collected extracts is adjusted to 25 mL in the mixing cylinder using DCM.

The specific masses for oil concentrations in the standards are determined as volumes of oil/dispersant solution multiplied by the concentration of the stock solution (mg/mL). An example standard calibration curve is given in Table 1.

Calibration point	Size of Volumetric (mL)	Stock volume used (μ L)	Theoretical concentration of oil in calibration (mg/mL)
0	25	0	0.000
1	25	20	0.055
2	25	40	0.110
3	25	60	0.165
4	25	80	0.220
5	25	100	0.275

Table 1. Example of a standard 5-point calibration curve, assuming a stock concentration of 68.79 mg/ml.

3.3 Sample preparation and Testing

For Type 2 testing, six replicates of oil and test dispersant plus one additional test of method blanks (artificial seawater without oil and dispersant) are used as a test set.

The 150 mL test flasks are set up with 120 mL of artificial seawater using a measuring cylinder and 400 μ L of oil. The dispersant is diluted 10 times with artificial seawater and 160 μ L of dispersant are added to the test flask to give a DOR of 1:25.

For Type 3 testing (neat) the test set comprises six replicates of oil and test dispersant, plus one additional test of method blanks (artificial seawater without oil and dispersant).

The 150 mL test flasks are set up with 120 mL artificial seawater using a measuring cylinder, 400 μL of oil and 16 μL of dispersant to give a dispersant to oil ratio (DOR) of 1:25. The dispersant is added undiluted.

The test flasks are shaken for 10 ± 0.25 minutes at 200 ± 10 rpm. After shaking flasks are set aside for 10 ± 0.25 minutes to allow undispersed and/or re-coalesced oil droplets to refloat to the surface. This part of the process requires exposure, settling and sampling time to be tightly controlled to reduce variability between replicates and blanks. This can be achieved by running each replicate separately, or by working with several people carrying out the same process on different replicates.

Following settling 30 mL of the water-phase are collected into a separation funnel and extracted 3 times by shaking each time with 5 mL of DCM added for each extraction. The extracts were combined in a volumetric flask and adjusted to a final volume of 25 mL.

3.4 Spectrophotometric Measurement and Calculations

The UV-Visible Spectrophotometer Linear Stability is calibrated daily for each oil/dispersant mixture individually. The instrument is adjusted using DCM as a blank reading for wavelength between 340 and 400nm. Samples and standards are transferred into screw-cap 10mm path cuvettes and the intensity measured at room temperature.

A blank sample must be run with each plate to blank correct the data prior to exporting.

Following analysis, the area under the curve is calculated using the Trapezoidal rule:

$$\int_{340\lambda}^{400\lambda} f(x)dx \approx \frac{H}{2} \sum_{k=1}^N (f(x_{k+1}) + f(x_k)) \quad (2)$$

Where $N+1$ = number of absorbance measurements to delineate N equally spaced sections of the curve, and H = the distance (λ) between each reading.

For $H = 5$, $N+1 = 13$ measurements. The following formula illustrates readings taken every 5λ .

$$Area = \frac{(Abs_{340} + Abs_{345}) * 5}{2} + \frac{(Abs_{345} + Abs_{350}) * 5}{2} + \dots + \frac{(Abs_{395} + Abs_{400}) * 5}{2} \quad (3)$$

After determining the area count for each standard, the response factor (RF) can be calculated for the standard at each concentration using the following equation:

$$RF = \frac{\text{Theoretical Concentration, } \frac{g}{mL} \text{ (Eq.1)}}{\text{area (Eq.3)}} \quad (4)$$

Instrument stability for the initial calibration is acceptable when the RFs of the five standard extracts are less than 10% different from the overall mean value for the five standards, as calculated in Equation 5.

$$\% \text{ difference} = \frac{|RF - \overline{RF}|}{\overline{RF}} * 100 \quad (5)$$

The slope of the calibration curve is determined using linear regression with a forced zero intercept

$$Y(\text{concentration of oil}) = \frac{X(\text{area under absorbance curve})}{m(\text{slope})} \quad (6)$$

The Total Oil dispersed and the percentage of oil dispersed (%OD) are calculated based on the ratio of oil dispersed in the test system to the total oil added to the system; using the following equations:

$$\text{Total Oil Dispersed, mg} = \frac{\text{Area (Eq.2)}}{\text{Calibration Curve Slope}} * V_{DCM} * \frac{V_{tw}}{V_{ew}} \quad (7)$$

Where: V_{DCM} = final volume of the DCM extract, mL

V_{tw} = total seawater in Baffled Flask (120 mL)

V_{ew} = volume seawater extracted (30 mL)

and

$$\%OD = \frac{\text{Total Oil Dispersed}}{\rho_{oil} * V_{oil}} * 100 \quad (8)$$

Where: ρ_{oil} = density of the specific test oil, mg/mL and

V_{oil} = Volume, mL of oil added to test flask (100 μ L = 0.1 mL)

If outliers are detected ($p < 0.05$), an additional replicate should be analysed to obtain the required six replicates.

3.5 Report

The overall %OD is the average of the six separate determinations. The calculated average is reported to one decimal place.

4 Test Validity

For the described test to be considered valid the following criteria must be met:

- i. at least 5% of all UV-visible spectrophotometric measurements should be done in duplicate as a QC check on the analytical measurement method with an agreement of absorbance values within $\pm 5\%$ of their mean value.
- ii. At least two method blanks should be analysed per completed test. The blank concentration is acceptable if the oil concentrations in the method blanks is less than detectable limits (spectrophotometric area count ≤ 2.5).
- iii. Accuracy must be tested by using a mid-point standard calibration check after each set of replicate samples analysed. The acceptance criterion is based on a percent recovery of 90-110% using the following equation:

$$\% \text{recovery} = \frac{\text{measured concentration in check standard}}{\text{theoretical concentration of check standard}} * 100 \quad (10)$$
- iv. Before sample analysis, instrument stability has been tested for each calibration. The instrument stability for initial calibration is considered acceptable when the RFs (Eq. 5) for each of the five standard concentration levels is less than 10% different from the overall mean value.
- v. Linearity – The regression co-efficient (r^2) of the analytical calibrations must be ≥ 0.99 .

5 Pass/Fail Criteria

A dispersant is considered efficacious if it meets the following criteria:

TEST No	TEST	TYPE 1	TYPE 2	TYPE 3	METHOD
1	APPEARANCE	CLEAR AND HOMOGENEOUS, - VISUAL INSPECTION			
2	DYNAMIC VISCOSITY AT 0°C (mPa s maximum)	50	250	250	ASTM D445 IP 71 BS 4708
3	FLASH POINT (°C minimum)	60	60	60	ASTM D93 IP 34 BS 2839
4	CLOUD POINT (°C maximum)	-10	-10	-10	ASTM D2500 IP 219
5	<u>Percentage of Dispersed oil (%)</u>	30*	30*	60*	This method
6	MISCIBILITY WITH WATER	N/A	Pass	N/A	At a ratio of 1:10 the dispersant:water mixture must not be more viscose that the dispersant alone

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