CHARACTERIZATION OF OIL-MINERAL AGGREGATES

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ABSTRACT: Oil associates with fine mineral particles in an aqueous medium not only as molecules adsorbed on mineral surfaces, but also as a discrete phase to form microscopic oil-mineral aggregates (OMA). On the basis of recent studies, OMA formation now is believed to be instrumental in the natural recovery of oil spill impacted shorelines and in the efficacy of cleanup techniques such as surf washing. A better understanding of the nature and properties of OMA will help predict the fate of oil spilled in the aquatic environment.

This work describes the various instruments and methods currently available for the detection and identification of OMA. Three types of OMA have been characterized by microscopy techniques: droplet, solid, and flake aggregates. Droplet aggregates are oil droplets (usually a few μm in diameter) surrounded by individual or aggregated mineral particles. Solid aggregates are a mixture of oil and minerals blended into microscopic bodies of various shapes. Flake aggregates are thin sheets reaching several millimeters across in which mineral and oil are arranged in a regular pattern.

Energy from breaking waves facilitates OMA formation. Once formed, OMA appear to be very stable structures the buoyancy of which depends on the ratio of oil to mineral in each individual aggregate.

Introduction

Oil-mineral aggregates (OMA) are microscopic entities composed of distinct oil and mineral phases that are stable over periods of weeks in seawater. Laboratory experiments by Bragg and Yang (1995) suggested that the formation of “clay-oil flocs” (microscopic agglomerations of oil droplets coated with mineral particles) might be instrumental in natural cleaning of oiled shorelines from the Exxon Valdez oil spill. In recent years, the presence of OMA at oil spill sites was confirmed by microscopic observation of nearshore water samples (Lee et al., 1997, 1999). Oil-mineral interactions are now not only believed to be instrumental in the natural removal of stranded oil within the coastal environment, but also in the effectiveness of surf washing as an oil spill countermeasure (Owens, 1998; Wood et al., 1997). This last hypothesis was confirmed during the Svalbard Shoreline Field Trials: mineral-bound oil concentrations in nearshore waters increased sharply immediately after the oiled sediments were relocated into the surf zone (Lee et al., 1999). Moreover, it has been shown that that oil biodegradation is enhanced in OMA (Lee et al., 1997; Weise et al., 1999). Widespread oil-mineral interactions on oiled shores is hardly surprising since OMA have been generated easily in the laboratory by shaking a variety of crude oils with seawater containing mineral particles (Lee et al., 1998; Weise et al., 1999; Wood et al., 1998), including oil and sediment from past spills (Bragg and Owens, 1994, 1995).

As oil-mineral interactions emerge as a potentially important factor influencing the fate of oil in the marine environment, oil spill responders must be able to establish whether OMA are forming at an oiled shoreline. This paper reports the current techniques used to observe and identify OMA and describes the various aggregate structures that commonly are observed in the field and in the laboratory.

Definitions

Aggregate. In colloid chemistry, the agglomeration of particles or colloids in suspension is termed aggregation. Aggregation caused by simple ions in solution is called coagulation whereas aggregation caused by larger molecules and polymers is termed flocculation (Lagaly, 1993). In the case of oil-mineral interactions, both mechanisms may coexist; thus, the term aggregate, rather than floc (Bragg and Yang, 1995), will be used in this paper.

Clay. In sedimentology, the term clay refers to the finest size fraction of sediment composed of particles less than 4 μm in size (Guggenheim and Martin, 1995), without reference to the chemical composition of the particles. In mineralogy, the clay minerals are composed mostly of alumino-silicates called the phyllosilicates because the majority crystallizes in sheet-like structures. Clay minerals are most abundant in the finer size fraction of sediment, although individual crystals can be larger than 4 μm. As mineral particles larger than 4 μm are incorporated into OMA, including minerals other than the phyllosilicates (e.g., quartz, Lee et al., 1998), the term mineral rather than clay will be used in this paper. “Mineral aggregates” will be used to designate aggregates of mineral particles only (no oil), and “clay minerals” is used when referring to the phyllosilicates (e.g., kaolinites, smectites, illite/mica, chlorites), as opposed to other minerals such as quartz, feldspars or calcite.

Oil. Oil refers to petroleum hydrocarbons as a distinct physical phase, in quantities exceeding their solubility in water. In other words, OMA contain a discrete oil component, in addition to oil adsorbed on mineral particles at the molecular level.

Methods

Because most OMA are well below 1 mm in size, their study relies almost exclusively on microscopy using bright field
transmitted light, UV epi-fluorescence, scanning confocal laser, and scanning electron beams.

**Sample processing.** Seawater samples taken for OMA analysis stored refrigerated will not degrade significantly over several weeks provided that they are fixed with mercuric chloride (approximately 200 ppm) to inhibit biodegradation.

Most seawater samples containing OMA must be concentrated before examination. In this study, 20 ml of well-homogenized sample was transferred to a 22-ml glass sampling vial 4.5 cm in height. After a 18-hour (overnight) settling period in the refrigerator, a known volume of water was aspirated from the center point of the liquid phase in the sample vial with a volumetric pipette, care being taken to preserve the floating phase gathered at the air/water interface and the sinking phase on the bottom of the vial. The volume withdrawn was chosen to achieve concentration factors of 3 to 60, depending on the sample (i.e., withdrawing 15 ml from the 20-ml sample results in a fourfold concentration factor). The guiding principle in choosing the concentration factor is to have on the slide the greatest number of particles in the vicinity of the oil, the UV light path can be blocked to eliminate oil fluorescence (Figure 1b).

**Combined transmitted light/UV epi-fluorescence microscopy.** UV epi-fluorescence illumination enables one to distinguish the oil phase because of the intense fluorescence characteristic of aromatic hydrocarbons. The non-fluorescent mineral particles can be observed simultaneously by applying a small amount of transmitted light (Figure 1a). To highlight the mineral particles in the vicinity of the oil, the UV light path can be blocked to eliminate oil fluorescence (Figure 1b).

To mount the sample, 30 μl of concentrated sample are aspirated with a micropipette from the sample vial on a vortex shaker at low speed, which keeps the particulate material in suspension, thus avoiding sampling bias due to sedimentation. The aliquot is deposited on a glass slide (25 x 75 mm) and covered with a glass slip (22 x 22 mm). The cover slip must be sealed at the edges (e.g., with nail polish) if oil immersion objectives are used. In this study, six transects spanning the width of the cover slip and approximately 2.5 mm apart were scanned with a Leitz Orthoplan microscope fitted with a combination of transmitted light and UV epi-fluorescence illumination (band pass excitation filter: 340–380 nm; reflection short pass filter: 400 nm; long pass suppression filter: 430 nm). Survey scans were done at a magnification of 160 × and photographs were taken at 160, 400, or 1,000 × in transmitted light mode, which shows mostly the minerals phase (Figure 1b), and in transmitted light plus epi-fluorescence illumination, which highlights the fluorescent oil (Figure 1a). The suggested fluorescence filter combination used seems to least affect the natural colors of the particulate matter in the sample while the oil fluorescence is bright and easily detected. However, as oil fluoresces over a wide range of wavelengths, other filter combinations can be used for OMA studies. For example, a band pass excitation filter of 450–490 nm, a short pass reflection filter of 510 nm, and a long pass suppression filter of 515 nm were effective but resulted in an overall yellow appearance of oil and particulate matter.

**Semiquantitative results may be obtained using a calibrated eye piece graticule to measure fields in the microscope, and a haemocytometer slide having a known sample thickness (100 μm) within two calibrated areas to calculate the sample volume in which OMA are counted. Moreover, the distance between the sinking aggregates, resting at the bottom of the counting cells, and the floating aggregates, against the cover slip, is large enough that the two can be observed separately by focusing in the two different planes. A micropipette is used to add 30 μl of well-mixed sample on each of the two counting chambers regions of the haemocytometer prior to placement of the cover slip. This method is preferred over the traditional method of filling the counting areas with the cover slip in place because large mineral

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**Figure 1.** Droplet oil-mineral aggregate as seen in simultaneous uv epi-fluorescence and transmitted light illumination (a) and transmitted light only (b). A mineral aggregate can be seen at the bottom of the photomicrographs. Scale bar: 20 μm.
grains or aggregates prevent uniform, unrestricted capillary flow of the sample into the counting cells. In the authors' laboratory, an automated method based on image analysis of a number of microscope fields of view has been developed to quantify the amount of oil present as OMA in a water sample.

Confocal scanning laser microscopy. Confocal scanning laser microscopy was used to elucidate the structure of OMA because the optical slicing capability of the instrument eliminates light originating from above and below a very narrow plane of focus. The samples are prepared exactly as for regular UV epi-fluorescence microscopy. The internal structure of the OMA was visualized with a Zeiss LSM410 confocal scanning laser microscope equipped with a Kr/Ar laser source (simultaneous excitation wavelengths of 488 and 568 nm) and an Axiovert 100 inverted epi-fluorescence microscope (60 × oil immersion objective lens). The signal emitted in the range 515–540 nm was recorded in the green channel for 10 to 15 scans in focal planes 2 µm apart, whereas all wavelengths above 515 nm were recorded in the red channel. The green channel represents the fluorescent signal of the oil phase (Figure 2a), and the most intense signal in the red channel is the reflectance from mineral particles (Figure 2b).

Environmental scanning electron microscopy. Electron microscopy, like confocal microscopy, is not a routine method but its high depth of field and magnification capabilities reveal the 3-dimensional appearance of OMA. The Environmental Scanning Electron Microscope (ESEM: ElectroScan E3, now Philips Electron Optics) operates with a partial pressure of water vapor in the specimen chamber, thus volatile substances such as petroleum hydrocarbons can be viewed without ill effects to the instrument. Moreover, if the sample is kept cold (a Peltier-effect cooling stage in this case), the sample can be kept wet if the water vapor pressure is selected appropriately.

In this study, the concentrated sample was diluted with filtered seawater (20 µL in 500 µL) and filtered onto a 12-mm diameter, 0.2-µm pore size polycarbonate filter (Millipore®-MF). The dilution was necessary to isolate OMA on the filter surface for analysis of their structure and can be adjusted for samples of different concentrations. The unrisned filter immediately was deposited over a bead of deionized water (to avoid dehydration during initial pump down of the specimen chamber) on the cooling stage of the ESEM and examined at 2°C, 500 Pa of water pressure to enable observation of a wet sample. The water still surrounding OMA helps preserve their shape and contrasts well with the darker oil, although a thin film of water blurs the surface details of the mineral phase (Figure 3a). Subsequently, the water pressure was lowered slowly until the water coating or surrounding the aggregates evaporated (Figure 3b). While the micrographs in the dry state show aggregates that may have deformed or instances where oil has spread out, the minerals and their relationship to the oil become more visible, and magnifications much higher than those available in light microscopy can be achieved.

The structure of OMA

Microscopical examination of over 200 samples of OMA generated in the laboratory (Kepkay et al., 2000; Lee et al., 1998; Weise et al., 1999) and sampled at oil spill sites (Lee et al., 1997, 1999, 2001) revealed three different structural types of OMA: droplet, solid, and flake.

Droplet OMA. Droplet OMA are composed of one or more droplets with mineral particles attached to their surface only (Figure 1 and 3). Confocal microscopy shows that the droplets do not enclose mineral particles (Figure 2). In the electron microscope, some hollow casing of mineral particles or perfectly smooth oil patches conclusively demonstrate that the mineral fines form a coating on the surface of the oil droplet and are not within the oil. The size of the oil droplets can vary from less than 1 µm to tens of µm, the larger sizes being found as floating droplet OMA. The quantity of mineral attached to a droplet is highly variable, and usually less in buoyant OMA than in sinking ones. In many cases, mineral aggregates, as well as individual mineral particles, are attached to the oil drop. Occasionally, an oil droplet is attached to a single large mineral particle. Although single-droplet OMA are the most common, aggregates composed of as many as 10 oil droplets have been observed (Figure 4a).

Figure 2. Optical "slice" through the center of a droplet oil-mineral aggregate in the scanning confocal microscope. Oil is located at the center as a droplet (a = green channel: 515-540 nm) whereas the mineral particles are found only at the periphery of the oil (b = red channel: > 515 nm). Scale bar: 10 µm.
Figure 3. A droplet oil-mineral aggregate in the environmental scanning electron microscope. In the wet state (a), the oil can be seen as dark areas of discontinuity in the coating of mineral particles. In the dry state (b), the details of the mineral particles are revealed whereas the oil exhibits a very smooth surface. Scale bar: 20 µm.

These multiple-droplet OMA are identical to those already reported in the literature (Bragg and Owens, 1994; Bragg and Yang, 1995; Lee et al., 1997, 1998).

**Solid OMA.** Solid OMA exhibit particles within the UV fluorescent area of the sample (i.e., oil boundaries), regardless of the sample focus plane, as indicated by confocal photomicrographs. Most solid OMA are nonspherical with irregular fluorescent contours molded around mineral particles (Figure 4b). Under the electron microscope, solid OMA appear as smooth oil surfaces interrupted by emergent mineral particles. In other words, the shape of the oil in solid OMA is controlled by the shape of the minerals included in it, whereas in droplet OMA the minerals are arranged around the exterior of the oil droplets. The solid OMA often are elongated and curved or even branched, and their size varies widely and can reach 250 µm in length. Lee et al. (1998) first described solid OMA.

** Flake OMA.** To the naked eye, flake OMA look like membranes, usually floating or neutrally buoyant, which can be several mm wide. They have been observed mostly in laboratory tests with montmorillonite (a smectite, or expandable clay mineral, often a major component of bentonite) and recently with sediment rich in smectite (Lee et al., 2001). Under epi-fluorescence they appear as sheets that have can be folded or rolled (Figure 4c). Their microstructure is highly organized in a dendritic or feather-like structure. Experiments suggest that high shear strength (i.e., extended or faster agitation) tends to break or crumple flake aggregates. This results in more compact OMA with less buoyancy, which may look more like solid OMA. However, the “crumpled” flakes may be distinguished from solid OMA because they often exhibit fold-like structures or preferential orientation of the minerals they contain (visible with transmitted light microscopy).

Figure 4. The three types of oil-mineral aggregates as seen in combined uv epi-fluorescence/transmitted light microscopy: multiple droplet aggregate (a), solid aggregate (b) and flake aggregate (c). Scale bars: a and b: 20 µm; c: 100 µm.
Conclusions

Evidence acquired to date in the laboratory and from actual oil spills indicates that significant OMA formation may occur naturally. The most commonly observed OMA are the droplet type, which suggests that oil slicks must first be broken up by the surf, resulting in droplets that are then coated by suspended mineral particles that prevent recoalescence of the oil (Bragg and Owens, 1994; Bragg and Yang, 1995). Thus, more viscous oils are likely to require more shear energy to form OMA. This hypothesis was confirmed by laboratory experiments (Lee et al., 1998; Wood et al., 1998) where, at a constant shaking speed, the most viscous oils (>10,000 mPa.s) did not form significant amounts of OMA. However, the existence of other OMA types (solid and flake) suggests that oil does not necessarily have to be dispersed as droplets in water to associate with mineral particles.

Besides the oil, the two other necessary components in the system are the availability of mineral particles in the silt or clay size range (<63 μm) and the presence of hydraulic shear energy (i.e., wave action). However, as OMA formation has been observed in actual situations with breaking wave heights of less than 30 cm (Owens et al., 1995; Sergy et al., 1999), it appears that sufficient hydraulic energy would be achieved at most shorelines. Fine mineral particles are ubiquitous in most beach sediments, although they may be present only beneath a layer of coarser material on shorelines with moderate to strong wave action. Sediment relocation (surf washing), a procedure where oiled sediment is excavated and transported to the lower intertidal zone at low tide, may be an effective spill countermeasure technique (Lee et al., 1999; Sergy et al., 1999). This procedure exposes underlying fine sediments to the surf and facilitates the breakdown of stranded oil into droplets, thus increasing the chances of OMA formation.

The variety of OMA structures described in this paper indicates that their formation is influenced by many factors, one of which is mineralogy, as smectites (e.g., montmorillonite) appear to preferentially form flake aggregates. The oil:mineral ratio of OMA controls its buoyancy, which in turn influences how far the oil will be dispersed from the oiled shoreline by waves and currents. Although more research is needed to elucidate the factors controlling the formation of OMA, it must be recognized that oil-mineral interactions are ubiquitous and that OMA formation will influence the outcome of some shoreline cleanup techniques as well as the fate of the dispersed oil. Studies have shown that oil associated with fine mineral particles in nearshore water or marine sediments has little toxic effects and is biodegraded faster than in the absence of mineral particles (Lee et al., 1997, 1999; Weise et al., 1999). Based on this study of the structure of OMA, tools and techniques to assess the potential of this process on an oiled shoreline have been developed (Stoffyn-Egli et al., 2000). These techniques should help responders to make decisions regarding the use of this natural phenomenon, which could result in spill countermeasures less costly, less damaging to the environment, and faster than other conventional methods.

Acknowledgements

The Research and Development Fund of the Canadian Coast Guard supports this work.

References


Biography

Dr. Kenneth Lee is a research scientist with the Department of Fisheries and Oceans (Canada). His research focuses on the relationship between biogeochemical processes and the microbial transformation of contaminants in coastal environments. He has conducted research on the ecological impact and effectiveness of oil dispersants, bioremediation, surf washing, and phytoremediation.


