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Tar Balls from Deep Water Horizon Oil Spill: Environmentally Persistent Free Radicals (EPFR) Formation During Crude Weathering

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Abstract

Tar balls collected from the Gulf of Mexico shores of Louisiana and Florida after the BP oil spill have shown the presence of electron paramagnetic resonance (EPR) spectra characteristic of organic free radicals as well as transition metal ions, predominantly iron(III) and manganese(II). Two types of organic radicals were distinguished: an asphaltene radical species typically found in crude oil ($g = 2.0035$) and a new type of radical resulting from the environmental transformations of crude ($g = 2.0041\text{--}47$). Pure asphaltene radicals are resonance stabilized over a polyaromatic structure and are stable in air and unreactive. The new radicals were identified as products of partial oxidation of crude components and result from the interaction of the oxidized aromatics with metal ion centers. These radicals are similar to semiquinone-type, environmentally persistent free radicals (EPFRs) previously observed in combustion-generated particulate and contaminated soils.

INTRODUCTION

The Deepwater Horizon (DH) incident of April 20, 2010 resulted in $4.4\text{--}4.9 \times 10^6$ barrels of crude oil being released into the waters of the Gulf of Mexico.^{1–3} Particularly affected were the shores of Louisiana, Mississippi, Alabama, and Florida.^{2–4} Though the thick slick of oil

is no longer a great threat to the ecosystem, long-term consequences are hard to predict. Fifty percent of the oil spill has not been accounted for.² Tar balls being washed onto the shore is one of the long-term effects of the oil spill.

Weathering processes of the crude emulsions fundamentally change the composition and chemical properties of the crude oil. Emulsification and dispersion of the spilled crude is a key step toward degradation of oil residues. At the same time, these processes lead to the formation of tar balls and oil–mineral aggregates (OMA).⁵ In principle, elimination of the lighter fraction of the crude due to either vaporization or dissolution of the spill material enriches the remaining part in heavier, less-polar fractions of the crude. Lighter fractions of crude, with a boiling point below 250 °C, evaporate within the first 24 h in normal sea conditions, eventually enriching the crude residues in the heaviest fractions.^{5,6} In addition to vaporization and dissolution of some of the fractions of the spilled crude, oxidation is an important weathering process.^{6,7} Oxidation of crude oil by microbial digestion is one of the remediation pathways; however, emulsions enriched in resins and asphaltenes are much less prone to biodegradation.⁷ Photo-oxidation is a vehicle for the chemical transformation of the heavier crude remnants and photolysis on the liquid–solid interfaces and has been reported to affect polyaromatic components⁷ producing acidic and phenolic compounds.^{6,8} As a result, weathered material can be more toxic to the ecosystem compared to unweathered emulsions.^{6,8}

The research on the ecological and toxic effect of oils spills is concentrated around the toxicity of the water-soluble PAH and other aromatic compounds.⁹ The heavier and insoluble fractions of the crude oil, as in tar balls, has not been considered a significant environmental threat except as a coating agent of animals and plants surface (birds, turtles, shore flora).¹⁰ In the case of sea turtles, tar ball ingestion and blockage of oral cavities have been reported.¹¹

The formation of tar balls and OMA during the emulsification and weathering of spilled oil results in adsorption of aromatic molecules and their chemical interaction with minerals. Since the suspended minerals and sediments contain crystallites and domains of transition metals,^{12–14} the metal–crude interaction is inevitable. Our recent studies of the surrogates of combustion borne particulate matter containing metal oxides have shown aromatic molecules chemisorb on the metal ion centers to produce surface stabilized environmentally persistent free radicals (EPFRs).^{15,16} During this reaction, chemisorbed species form surface, metal–molecule complexes, which undergo an electron transfer process resulting in surface-bound organic radicals. The formation of the radicals upon the interaction of the aromatic molecules with the transition metal center is an unexpected result by itself. What is more important is that radicals formed this way are persistent in the environment. Studies have shown that once formed, EPFRs are stable in the air for a prolonged time and their half-lifetimes in air are on the order of hours and days (formed over CuO/ silica and Fe₂O₃/ silica systems, respectively).^{15,16} Such long lifetimes of the radicals are unique; however, they do not preclude radical reactivity. The mechanism of the EPFR formation from interaction with metal ions has been shown to occur at elevated temperatures in combustion systems, but also in ambient conditions such as in soils at contaminated Superfund sites.¹⁷

Thus, study of the formation of stabilized radicals in crude remnants appears warranted. Their presence can have a critical impact on the affected ecosystems, as EPFRs have been shown to be potent reactive oxygen species (ROS) generators in aqueous media.¹⁸ The objective of the present study is twofold: identification/detection of the organic free radicals and transition metals in tar balls and investigation of EPFR changes that occur with time in the environment. All experiments presented were performed using samples collected in the Gulf of Mexico following the DH incident.

EXPERIMENTAL SECTION

Tar Ball Samples

The tar balls used for this study were collected from the Gulf of Mexico shores at four different times after the DH Gulf of Mexico oil spill. The samples were labeled as TB₀, TB₆₀, TB₉₀, and TB₄₅₀, with the subscript representing the number of days from the incident when collected (cf. Table 1). Each sample was analyzed primarily for the presence of paramagnetic signal, either from metal centers or organic radicals.

The kinetic studies were performed to determine the persistency and stability of the radicals in air. The samples were exposed to ambient air and EPR spectra were obtained regularly to determine the concentration of radicals as a function of time (cf. Table 1). Radical decay kinetic experiments in air are based on an assumed reaction with molecular oxygen:

For reaction rate calculations, a first order kinetic expression was used, $-dR/dt = k[R]$, where $k = K[O_2]$ for excess oxygen conditions. The 1/e half-life decay is calculated from the first order decay $t_{1/e} = 1/k$, where k is a slope of the linear regression to the integrated kinetic differential equation $\ln(R/R_0) = -kt$.

Radical Extraction

To study the origin of the components of complex paramagnetic signals, tar ball samples were extracted from the matrix using polar and nonpolar solvents, isopropyl alcohol and *tert*-butyl benzene, respectively. A small quantity of each sample was placed in extracting vials and sonicated with 2 mL of solvent for 1 h. The extract and the residue were separated by centrifuging for 10 min, upon which the solids were dried in the oven for 2 h at 37 °C. The liquid part was introduced into capillary tubes, sealed with critoseal, and then placed in high purity quartz EPR tube.

EPR Measurements

The samples were analyzed for the presence of EPFRs without any prior modifications by placing them into 4-mm ID, suprasil EPR tubes. The spectra were measured using a Bruker model EMX 10/2.7 spectrometer at room temperature. The instrumental conditions were as follows: microwave frequency (9.5 GHz), microwave power of 2 mW, center field at 3450 G, sweep width of 200 or 1000 G, resolution 1024 points, receiver gain of 1×10^4 , modulation frequency of 100 kHz, time constants of 40 ms, and sweep width of 167 s. A

di(phenyl)-(2,4,6-trinitrophenyl)-iminoazanium (DPPH) standard was used to calibrate both the field position and radical concentration.

The assignments of the spectral components in the resulting EPR spectra were preformed based on the spectral deconvolution using the Origin 7E Peak Fitting module to obtain a best fit (0.999 or higher with minimal number of peaks). The fitted absorption spectrum was compared with the original first-derivative and absorption spectra. The spins per gram (concentration) were calculated based on comparison to a DPPH standard.

OH Radical Generation Studies

Due to a short lifetime of the OH radicals (up to 10^{-9} s) a special technique is required. To study the potential of OH radical formation from tar balls in aquatic environment spin trapping experiments of OH were performed using 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) molecule as a spin trap and the EPR spectra of DMPO–OH adducts were recorded. The spin-trapping process takes advantage of the stability of the nitroxyl radicals and is based on a reaction between the spin trap molecule and OH radical to produce a stable paramagnetic aminoxyl (or nitroxide) species, which are referred to as a spin adduct (cf. Scheme 1). Thus formed aminoxyl product has a unique EPR spectrum. A 1–1 stoichiometry between DMPO and OH radical is assumed for this reaction, DMPO–OH adduct corresponds to one mole •OH in solution. High purity 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) was obtained from ENZO Life Sciences International and was used without further purification. An appropriate amount of tar ball samples was homogenized in a phosphate buffer solution (PBS, pH = 7.4) to obtain a uniform suspension containing 100 µg/mL of particles. Next, 10 µL from a freshly prepared solution of 150 mM solution of DMPO was added to the 100 µL of the tar ball suspension and the final solution balanced to 200 µL with PBS. The EPR spectra were measured periodically at different time intervals resulting in 4-lines characteristic peaks of DMPO–OH. The area of the two inner peaks was determined by double integration using Bruker WINEPR system, Version V2.22 Rev.10.

RESULTS AND DISCUSSION

Sample Characterization and Physical Appearance

The samples, based on their physical appearances, could be divided into 2 categories: hard, black, glossy material and soft, brown material. The latter is consistent with a typical appearance described in literature of tar balls containing weathered crude remnants, sand particles, and biodebris. Samples TB₆₀, TB₉₀, and TB₃₆₀ belonged to this category. One sample, TB₀, had a black and glassy appearance. Interestingly, this sample was collected at the very early stages of the DH incident, when the oilrig platform was still burning. A distinct difference of the appearance of the sample TB₀ compared to TB₆₀, TB₉₀, and TB₄₅₀ indicates a major chemical transformation upon release to the environment.

Sample TB₀—The physical appearance of the TB₀ sample (black, hard, and glassy) suggested the sample was a solidified pure asphaltene material. The solubility test of the samples was consistent with initial assumption, as the sample was completely dissolved in *tert*-buthylbenzene (no residue left) and nonsoluble in polar solvents or *n*-alkanes (isopropyl

alcohol, *n*-hexane).¹⁹ The EPR spectra of TB₀ revealed the presence of a single, symmetric, and strong paramagnetic signal (cf. Figure 1) centered at 3454 G, characterized by a *g* value of 2.0035 and the *H*_{p-p} of 5.1 G. The parameters of the spectrum recorded were consistent with the radical species observed in the crude oil and originating from asphaltene species.^{20,21} The spin number of detected radicals (6.7×10^{18} spins/g of sample) was in the range of the number of radicals present in pure asphaltenes.²² Unlike other reports, no spectral features were observed typical for the presence of metal centers, often present in asphaltenes from crudes.^{23,24} No spectral features characteristic to metal centers was only an indication of nonpresence of those metals in oxidation states with a paramagnetic electron. Reduction or oxidation of metal ions due to the interaction with organic material can result in disappearance of the metal-associated EPR signal.

The nature of the radical species in asphaltenes is not clearly understood. Unpaired electrons are thought to be delocalized across the aromatic π system stabilized by resonance on the intrinsic polyaromatic sheets of asphaltene molecule.²¹ This resonance stabilization of the observed radicals in asphaltenes contributes to their high persistency, as they remain unchanged almost indefinitely. No changes were observed in the spin numbers of the collected sample, even after 2 years of exposure to ambient air. The mechanism of the radical formation in asphaltenes molecules is unknown. Some studies have indicated the correlation between the paramagnetic signal and the number of transition metals (vanadyl groups in particular); however, studies are inconclusive.^{24,25}

Tarballs TB₆₀, TB₉₀, and TB₄₅₀—Tar balls collected 60, 90, and 450 days after the DH incident exhibited a much more complex EPR spectrum compared to that of TB₀ (cf. Figure 2), indicating the presence of multiple organic radical species and paramagnetic metal ions.

For each of those samples a distinct organic radical signal was recorded at *g* ~ 2.0041 and with a *H*_{p-p} ranging from 5.2 to 5.7 G (cf. Figure 2A). While the width of this signal was increased only slightly, its position was distinctly different from the one observed for sample TB₀. The broadening of the spectrum was an indication of more than one radical contribution to the overall spectra. Superposition of the signals of different species was further supported by the lack of symmetry of the spectra. The shift in *g* value from 2.0035 to 2.0041 suggested the presence of more oxygen-centered radical species.^{17,26–28}

In the broad magnetic field of 1000 G several other paramagnetic species were also detected, not observed in TB₀ sample (cf. Figure 2B). Lines detected at *g* = 2.5 and *g* = 4.7 can be assigned as originating from paramagnetic Fe³⁺ ions—mutually interacting Fe³⁺ ions and Fe³⁺ ions in tetrahedral coordination, respectively.^{29,30} Theoretical calculations have demonstrated the *g*-value at 3.3 to represent Fe³⁺ in glassy environment.³¹ Weak EPR lines in TB₉₀ at *g* = 2.00 and *g* = 4.3 are characteristic for Fe³⁺ in a distorted tetragonal site, where Fe³⁺ ions substitute for Si⁴⁺ in SiO₂ structures.³² In the case of TB₄₅₀, a six-line hyperfine structure characteristic to the interaction of Mn(II) ions with its nuclear spin (*I* = 5/2)^{17,33} was also observed.^{17,33}

Sample Aging and Radical Decay

Unlike TB₀, the concentration of the organic paramagnetic signals in samples TB₆₀, TB₉₀, and TB₄₅₀ decreased upon exposure to air outside the marine environment (cf. Figure 3), with a calculated 1/e half-life of 400–600 days. Slow radical decay further underlined the differences between the asphaltene radical and the tar ball radicals. High delocalization of the paramagnetic electron in the polyaromatic π system of asphaltenes results in indefinite stabilization by multi-ring resonance. As a result, asphaltene radicals are nondegrading and are resistant to environmental oxidation. A gradual radical decay of radicals in TB₆₀, TB₉₀, and TB₄₅₀ partially revealed the multicomponent structure of the overall spectrum. One of the components of this spectrum at $g = 2.0017$ appears to be Fe³⁺ ions, which were also detected in a pure sand sample from the Gulf of Mexico beach (cf. Figure 3) with the remaining spectral components originating from the organic species.

The extraction of the samples with polar (isopropyl alcohol -IPP) and nonpolar (*tert*-butylbenzene- TBB) solvents resulted in a complete dissolution of the TB₀ in TBB (no residue remaining), and only slight dissolution in IPP (cf. Figure 4). The EPR spectra of the TBB extract revealed the presence of a weak signal at $g = 2.0035$ similar to that observed in original tar ball solid. No radicals were detected in the IPP extract.

The extraction of TB₆₀ and TB₉₀ in TBB resulted in a distinct change in the EPR spectrum of the residue (cf. Figure 4) with the signal in the residue centered at $g = 2.0017$. The signal parameters and the physical appearance of the residue indicated the remaining part to be sand particles incorporated in tar ball. Unlike the case of TB₀, no paramagnetic signal was detected in the extract, indicating the lack of self-stabilization of the radicals in these 3 tar ball samples. Extraction of the TB₆₀, TB₉₀, and TB₄₅₀ samples with IPP resulted in a decrease in the radical concentration with no change in overall g -value in the solid residue and no radical signal in the extract.

Tar Ball Radicals

Both TB aging and extraction have provided details on the components of the paramagnetic signal of TB₆₀, TB₉₀, and TB₄₅₀. As observed for sample TB₀ and indicated in literature, the g values of asphaltenes range from 2.0028 to 2.0035 and are associated with carbon centered, ring delocalized radicals.^{20,21,34} Higher g values were observed for TB₆₀, TB₉₀, and TB₄₅₀ samples ($g = 2.0041$) and are indicative of the presence of oxygen-centered radicals. In fact, the ability to extract a significant portion of the radicals with IPP is consistent with the enhanced polarity of the species containing oxygen atoms. Such extractability was not detected in the case of pure asphaltene samples. The disappearance of the radical signal in the extract for TB₆₀, TB₉₀, and TB₄₅₀ samples indicated the radicals in the tar balls were not stabilized and reactive when removed from the tar ball system.

The formation and stabilization of oxygen centered radicals on the solid particles in the environmental samples may result from the interaction between the metal cations and aromatic molecules. Such phenomena has been observed and described for both combustion-generated particulates and in the soil samples.^{17,26–28} We have previously demonstrated the formation of EPFRs on metal surfaces of Cu(II)O/silica²⁶ and Fe(III)O/

silica²⁷ particles leading to the reduction of metal oxide via electron transfer and formation of phenoxyl or semiquinone-type radicals. A similar mechanism may occur in the tar ball samples, where a presence of Fe³⁺ ion centers was detected. Using EPR spectral deconvolution procedures developed for metal–radical adducts,^{26–28} we were able to assign the *g* values to the components of the observed spectra of TB₆₀ and TB₉₀ (cf. Table 2). The presence of the Fe³⁺ ion centers (*g* = 2.0015–2.0017) have been shown for both pure sand and, upon a complete extraction of the tar ball samples, indicating association of iron ions with sand in the tar balls. A component at *g* = 2.0033–2.0034 is typical for the carbon-centered organic radicals and is most likely associated with the presence of asphaltene structures native to crude. The last component at *g* = 2.0047–2.0049 is typical to the semiquinone radicals bound to the reduced metal ion center. These radicals in a semibonded state have singlet line signals and are stabilized by the metal center against oxidation and decomposition.^{27,28}

Weathering of the spilled crude in the marine environment results in incorporation of sediments in the crude material and adsorption of organic matter to sand particles. The presence of iron in the sand particles resulted in the direct interaction of the adsorbed species, partial oxidation and formation of the surface stabilized radicals. This mechanism appears to be similar to the observed formation of EPFRs in the soils contaminated with pentachlorophenol (PCP) in the ambient environment.¹⁷ It has been shown that exposure of soils to pentachlorophenol result in the formation of radical species characterized by a singlet EPR spectrum with *g* value of 2.0031 and peak width of 6 G.¹⁷ These radicals are formed by an electron transfer reaction between the metal ion center in soil and adsorbed PCP molecule, resulting in primarily keto form of pentachlorophenoxy radical, due to the electronic effect of 5 chlorine atoms on benzene ring (hence, the lower *g* value). In fact the presence of 6000–7000 ppm of PCP in soil resulted in 2×10^{18} radicals per g of soil. In the case of tar balls the concentration of the radicals is at the level of 3×10^{17} radicals/g of tar ball. Though the concentration is an order of magnitude lower compared to contaminated soils, it is surprisingly high, considering the fact that these radicals are formed from ambient condition of oxidation of residual organic matter, not by exposure to the reactive PCP contaminant. Interestingly, also the nature of the formed radicals is different, since tar ball EPFRs were identified as predominantly of semiquinone type.

Generation of Hydroxyl Radicals by EPFRs in Tar Balls

The formation of EPFRs in tar balls indicates chemical changes in the crude during the weathering process in the marine environment. Their presence in tar balls can have far-reaching environmental impacts: it has been shown before that EPFRs in the particulate matter are active in formation of superoxide radicals and hydroxyl radicals.^{18,35} Two tar ball samples have been tested for their OH radical generation potential based on the formation of DMPO–OH spin adduct: TB₀ and TB₄₅₀ (cf. Figure 5). We have selected TB₀ sample to serve as a reference for the weathered tar ball as it contains exclusively asphaltene radicals. difference in the OH radical yield between TB₀ and TB₄₅₀ is representing the change in the OH radical generation potency due to the formation of new radicals (EPFRs) resulting from weathering process. After only 1 h of suspending the tar balls in PBS solution (cf. Figure 5) TB₄₅₀ showed ~50% higher yield of DMPO–OH spin adduct and almost 2-fold higher

amount of DMPO–OH formed after 12 h of reaction. Since the total radical concentration in TB₀ is 200 times that of TB₄₅₀ ($\sim 6 \times 10^{18}$ and $\sim 3 \times 10^{16}$ spins/g, respectively) the overall yield of the OH radicals formation from EPFRs is ~400 times that of asphaltene radicals. Such high OH radical formation potential by EPFRs in tar balls indicates their high environmental hazard. On the other hand, asphaltene radicals in TB₀ are also capable of generating OH radicals, however, due to their intrinsic nature they are not readily available for the reaction to occur.

ENVIRONMENTAL IMPACT

In comparison with the other EPFR–particle systems, radicals found in tar balls have long 1/e half-lives and can remain in the marine environment for a very long time (cf. Figure 3). Surface bound radicals of both phenoxyl and semiquinone-type have been shown to be able to generate hydroxyl radicals through a cyclic chemical process without substantial decay of the parent EPFR system.^{18,36} The newly discovered EPFR radicals are “bound” to the tar-ball matrix and as such their mobility inside the matrix is limited. Thus, it is difficult to imagine them being directly involved in the transformation of the tar balls organic matter. However, they are a product of the weathering or oxidation of organic matter and in that sense they are active in tar ball transformation. Also, they can be active indirectly, for example, in generation of hydroxyl radicals, which in turn can react with the organic matter of tar balls contributing to a further oxidation process.

Both *in vitro* and *in vivo* experiments have indicated initiation of cardiopulmonary disease from the particles containing EPFRs.³⁷ Though the large tar balls do not pose a direct threat for inhalation or ingestion, small sand particles exposed to crude oil can also contain oil-born radical species. These small sand particles can be windblown, become airborne, inhaled, or digested.

Presented here is identification and discovery of the environmentally persistent free radicals in tar balls during weathering process which should only be the starting point of the research of this new species. It is of utmost importance to determine the environmental factors such as, pH, salinity, water oxygen saturation, etc., contributing to the formation of EPFRs as well as affecting their lifetime. Only then a full picture and understanding of the environmental effects of EPFRs in tar balls can be achieved.

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Scheme 1.
DMPO Spin Trapping of OH Radicals

Figure 1. EPR spectra of TB_0 at room temperature. (A) Spectrum at 200 G field and (B) at 1000 G field, respectively.

Figure 2. Spectra of TB₆₀, TB₉₀, and TB₄₅₀: (A) 50 G field spectrum of organic radical and (B) 1000 G field spectrum.

Figure 3.
Comparison of the EPR spectra of TB₆₀ and TB₉₀ while aging in air.

Figure 4.
EPR spectra of TB₉₀ before and after extraction with isopropyl alcohol and tert-butylbenzene.

Figure 5.
Formation of DMO-OH adduct in the tar ball suspension in PBS.

Table 1

Tar Ball Samples, Collection Location, and Designation

sample designation	collection location	collection date	no. of days since DH incident	post aging in air	no. of days post-aged
TB ₀	Gulf Shores, AL	05/01/2010	12	--	
TB ₆₀	Gulf Shores, AL	06/15/2010	56	--	
TB ₉₀	Gulf Shores, AL	07/21/2010	92	--	
TB ₄₅₀	Pensacola Beach, FL	07/14/2011	450	--	
TBA ₁₆₀	Gulf Shores, AL	06/15/2010	56	yes	232
TBA ₂₆₀	Gulf Shores, AL	06/15/2010	56	yes	409
TBA ₁₉₀	Gulf Shores, AL	07/21/2010	92	yes	196
TBA ₂₉₀	Gulf Shores, AL	07/21/2010	92	yes	373

Table 2

EPR Spectral Deconvolution of Tar Ball Samples

peak no.	position	TB ₆₀	TB ₉₀	structural assignment of radical
g1		2.0015	2.0017	Fe ³⁺ center
g2		2.0033	2.0034	delocalized carbon centered
g3		2.0049	2.0047	semiquinone type