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Efficient extraction and detection of aromatic toxicants from crude oil and tar balls using multiple cyclodextrin derivatives

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ABSTRACT

Herein we report the efficient extraction of aromatic analytes from crude oil and tar balls using multiple cyclodextrin derivatives. The known propensity of the cyclodextrins to bind hydrophobic guests in their hydrophobic interiors enhanced the extraction of aromatic analytes from the oil layer to the aqueous layer, with methyl-β-cyclodextrin and β-cyclodextrin providing the most significant enhancement in extraction efficiencies of aromatic toxicants (69% aromatic toxicants in aqueous layer in the presence of methyl-β-cyclodextrin compared to 47% in cyclodextrin-free solution for tar ball oil extraction), and provide optimal tunability for developing efficient extraction systems. The cyclodextrin derivatives also promoted efficient energy transfer in the aqueous solutions, with up to 86% efficient energy transfer observed in the presence of γ-cyclodextrin compared to 50% in the absence of cyclodextrin for oil spill oil extraction. Together, this dual function extraction followed by detection system has potential in the development of environmental remediation systems.

1. Introduction

Anthropogenic oil spills such as the Deepwater Horizon oil spill of 2010 highlight a number of unsolved problems in the areas of oil spill cleanup and remediation (Gohlke et al., 2011; Anastas et al., 2010; McNutt et al., 2012), efficient detection of oil-spill related toxicants in complex environments (Radovic et al., 2012), and the monitoring and understanding of long-term effects of oil spills on complex ecosystems (Yim et al., 2012). Current methods used for the cleanup of oil spills include skimming or booning of the oil (Broje and Keller, 2006), burning oil on the surface of the water (Sneddon et al., 2006), applying chemical dispersants to facilitate oil dispersion (Saha et al., 2013), and introducing oil-eating bacteria for environmental bioremediation (Yang et al., 2009). Many of these methods suffer from potentially serious drawbacks, including the environmental damage from oil burning (Prendergast and Gschwend, 2014), the unknown toxicity of many dispersants (Wise and Wise, 2011), and the long-term disruption to the ecosystem from the introduction of non-native oil-eating bacteria (Ahluwalia and Sekhon, 2012). In recognition of these problems, newer environmentally–friendly cleanup methods have been developed by several research groups, including the synthesis of new hydrophobic materials, including thermally reduced graphene, a sponge, and porous materials (Iqbal and Abdala, 2013; Wang and Lin, 2013; Peng et al., 2014).

We have developed a new approach for the cleanup of oil spills in marine environments that focuses on the removal of aromatic toxicants such as polycyclic aromatic hydrocarbons (PAHs) (Serio et al., 2013). The removal of PAHs is particularly important because many of these compounds are known carcinogens or pro-carcinogens (Jarvis et al., 2014), including the Class I carcinogen benzo[*a*]-pyrene (Chart 1, compound **3**) (Nebert et al., 2013). This approach uses commercially available, non-toxic γ-cyclodextrin to bind PAHs and extract them from complex oils. Following the extraction, the PAHs are detected using cyclodextrin-promoted energy transfer to a high quantum yield fluorophore (compound **4**); analogous energy transfer has already been established as an efficient method for toxicant detection in multiple complex environments (Serio et al., 2014; Mako et al., 2012; Serio et al., 2013, 2014). Other research groups have also reported the use of cyclodextrin derivatives to extract PAHs from complex environments, including from contaminated soil (Sanchez-Trujillo et al., 2013; Petitgirard et al., 2009) and river sediments (Schulze et al., 2012).

Previous research in our group focused on the use of γ-cyclodextrin for the extraction and detection of PAHs from motor oil, vegetable oil, and vacuum pump oil. Shortcomings of this method included the moderate extraction efficiencies observed using γ-cyclodextrin, as well as the use of commercially available oils rather than oils that had been collected from contaminated

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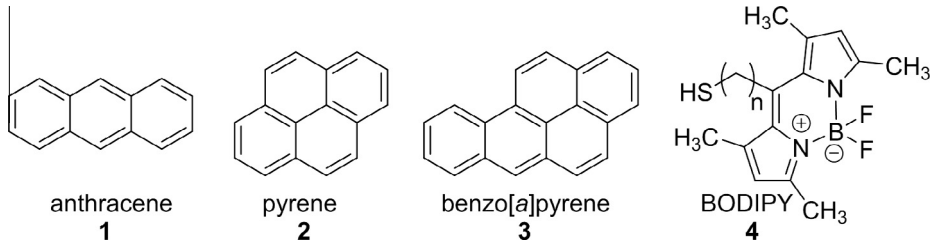

Chart 1. Structures of the analytes (**1–3**) and fluorophore **4** under investigation.

marine environments. Oil collected from oil spills (termed "oil spill oil") is more complex than the commercially available oils previously investigated, with a broad distribution of alkanes, aromatic compounds, and insoluble polymeric components (Wang and Fingas, 2006; Panda et al., 2007). These oils also contain many oxidized PAH derivatives as a result of the exposure of the oil to oxygen-rich environments (Filatov et al., 2013). Some crude oil spontaneously forms tar balls, which are oil-containing spheres formed from both oil spills as well as from naturally occurring oil sources (Hostettler et al., 2004). The degradation and oxidation of toxicants in tar balls has been shown to differ from that of toxicants found in bulk oil samples (Pendergraft and Rosenheim, 2014).

Reported herein is the use of a wide variety of cyclodextrin derivatives (α-cyclodextrin, β-cyclodextrin, methyl-β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin (2-HPCD), and γ-cyclodextrin) to extract and detect aromatic toxicants from motor oil, oil spill oil, and tar balls. The extraction and detection efficiencies depend both on the identity of the oil and on the cyclodextrin host. The aromatic small molecules extracted with cyclodextrin include highly toxic PAHs, polar oxidized PAH metabolites, and a variety of other toxicants that have been found in such complex matrices (Kang et al., 2014). The ability of cyclodextrin to extract multiple classes of toxicants simultaneously provides a significant operational advantage in the environmental remediation of polluted marine environments.

2. Materials and methods

2.1. Materials and methods

Three oil samples were analyzed: Pennzoil SAE-5W30 motor oil, oil collected from an oil spill site (collected in Louisiana, April 2012), and tar ball oil (collected in Alabama, November 2013). Polycyclic aromatic hydrocarbons (PAHs) **1–3** were purchased from Sigma Aldrich Company and were used as received (Chart 1). These PAHs were intentionally doped into the complex oil samples for the 'doped oil experiments' to measure the ability of cyclodextrins to extract and detect doped PAHs. Highly fluorescent compound **4** was synthesized following literature-reported procedures (Shepherd et al., 2004), and was used in the energy transfer experiments as a high quantum yield energy acceptor. Spectra/Por® 2 Dialysis membranes (Flat Width 45 mm, MWCO 12–14 kD) were purchased from Fisher Scientific and rinsed in deionized water for 15–20 min, in accordance with the product instructions. Fluorescence measurements were recorded on a Shimadzu RF5301 spectrophotofluorimeter, with a 1.5 nm excitation slit width and a 1.5 nm emission slit width. All spectra were integrated versus wavenumber on the *X*-axis using OriginPro software, version 9.1.

2.2. Preparing motor oil, tar ball oil, and oil spill oil for analysis

The motor oil was diluted with an equal volume of *n*-hexanes (1.25 mL of motor oil and 1.25 mL of *n*-hexanes). To prepare the oil spill oil, the oil was diluted in a 1:4 ratio with *n*-hexanes (0.625 mL of oil spill oil and 1.875 mL of *n*-hexanes). The tar balls were prepared by placing a tar ball (weighing ~1.50 g) in a mortar and pestle and breaking it up mechanically. Then, 5 mL of hexanes was added and the tar balls were mixed into the hexanes solution. The solution was then placed in a dialysis bag and placed in a beaker with approximately 400 mL of *n*-octane. The sample was allowed to dialyze for 3 days until the octane turned brown in color. After this time, the bag was removed and the resulting octane solution was centrifuged at 3000 rpm for 10 min. The brown solution was then decanted and stored as the tar ball extract solution. For each experiment, 2.5 mL of this stock solution was used.

2.3. PAH extraction techniques

2.5 mL of each oil sample (motor oil, oil spill oil, tar ball extract) was mixed with 20 μL of a 1 mg/mL solution of each analyte (**1–3**) in tetrahydrofuran (THF), or with 20 μL of pure THF (undoped sample). The samples were vigorously shaken by hand for 1 min, and the oil mixtures were then added to a 2.5 mL aqueous solution of either a 10 mM in phosphate buffered saline (PBS) cyclodextrin derivative (α-cyclodextrin, β-cyclodextrin, methyl-β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin (2-HPCD), and γ-cyclodextrin) or a 0 mM cyclodextrin solution in PBS (control). The mixture was vigorously shaken by hand for 1 min to ensure thorough mixing. The layers were allowed to sit undisturbed for 16–24 h. The layers were separated and the analytes in each layer, both the doped analytes (**1**–**3**) and the undoped samples, were detected by fluorescence spectroscopy with 360 nm excitation. The analyte fluorescence emission spectrum was integrated versus wavenumber on the *X* axis (using OriginPro 9.1 software). The amount of analyte in each layer was quantified as an "analyte comparison" and calculated according to Eq. (1):

$$\text{Analyte comparison} = I_{\text{aqueous}}/(I_{\text{aqueous}} + I_{\text{oil}}) \times 100\% \quad (1)$$

where I_{aqueous} is the integrated emission of the analyte in the aqueous layer and I_{oil} is the integrated emission of the analyte in the oil layer.

2.4. Energy transfer detection techniques

To a 2.5 mL solution of oil was added 100 μL of compound **4** (0.1 mg/mL in THF), 20 μL of the analyte of interest (1.0 mg/mL in THF) or 20 μL of pure THF ("undoped"), and 2.5 mL of aqueous solution (10 mM or 0 mM cyclodextrin derivative solution in PBS). The layers were vigorously shaken in a vial for 1 min and the layers were allowed to separate for 16–24 h. The layers were separated and each layer was excited at two wavelengths: the analyte excitation wavelength (360 nm) and the fluorophore excitation wavelength (460 nm). Each fluorescence emission spectrum was integrated versus wavenumber on the *X* axis (using OriginPro 9.1 software). The efficiency of the energy transfer from the analytes to the fluorophore was calculated according to Eq. (2):