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TITLE PAGE

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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.
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,\textsuperscript{1-3} efficient detection of oil-spill related toxicants in complex environments,\textsuperscript{4} and the monitoring and understanding of long-term effects of oil spills on complex ecosystems.\textsuperscript{5} Current methods used for the cleanup of oil spills include skimming or booning of the oil,\textsuperscript{6} burning oil on the surface of the water,\textsuperscript{7} applying chemical dispersants to facilitate oil dispersion,\textsuperscript{8} and introducing oil-eating bacteria for environmental bioremediation.\textsuperscript{9} Many of these methods suffer from potentially serious drawbacks, including the environmental damage from oil burning,\textsuperscript{10} the unknown toxicity of many dispersants,\textsuperscript{11} and the long-term disruption to the ecosystem from the introduction of non-native oil-eating bacteria.\textsuperscript{12} 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.\textsuperscript{13-15}
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).\textsuperscript{16} The removal of PAHs is particularly important because many of these compounds are known carcinogens or pro-carcinogens,\textsuperscript{17} including the Class I carcinogen benzo[a]pyrene (Chart 1, compound 3).\textsuperscript{18} This approach uses commercially available, non-toxic \(\gamma\)-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.\textsuperscript{19-22} Other research groups have also reported the use of cyclodextrin derivatives to extract PAHs from complex environments, including from contaminated soil\textsuperscript{23,24} and river sediments.\textsuperscript{25}

Previous research in our group focused on the use of \(\gamma\)-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 \(\gamma\)-cyclodextrin, as well as the use of commercially available oils rather than oils that had been collected from contaminated 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.\textsuperscript{26,27} These oils also contain many oxidized PAH derivatives as a result of the exposure of the oil to oxygen-rich environments.\textsuperscript{28} 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.\textsuperscript{29} The degradation and oxidation of toxicants in tar balls has been shown to differ from that of toxicants found in bulk oil samples.\textsuperscript{30}
Reported herein is the use of a wide variety of cyclodextrin derivatives (α-cyclodextrin, β-cyclodextrin, methyl-α-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. The ability of cyclodextrin to extract multiple classes of toxicants simultaneously provides a significant operational advantage in the environmental remediation of polluted marine environments.

MATERIALS AND METHODS

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, 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 to 20 minutes, 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.
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 \( \sim 1.50 \) 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 minutes. 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.

PAH extraction techniques. 2.5 mL of each oil sample (motor oil, oil spill oil, tar ball extract) was mixed with 20 \( \mu \)L of a 1 mg/mL solution of each analyte (1-3) in tetrahydrofuran (THF), or with 20 \( \mu \)L of pure THF (undoped sample). The samples were vigorously shaken by hand for 1 minute, 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 (\( \alpha \)-cyclodextrin, \( \beta \)-cyclodextrin, methyl-\( \beta \)-cyclodextrin, 2-hydroxypropyl-\( \beta \)-cyclodextrin (2-HPCD), and \( \gamma \)-cyclodextrin) or a 0 mM cyclodextrin solution in PBS (control). The mixture was vigorously shaken by hand for 1 minute to ensure thorough mixing. The layers were allowed to sit
undisturbed for 16-24 hours. 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 Equation 1:

\[
\text{Analyte comparison} = \frac{I_{\text{aqueous}}}{I_{\text{aqueous}} + I_{\text{oil}}} \times 100\%
\]  

(Eq. 1)

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

**Energy transfer detection techniques.** To a 2.5 mL solution of oil was added 100 \(\mu\)L of
compound 4 (0.1 mg/mL in THF), 20 \(\mu\)L of the analyte of interest (1.0 mg/mL in THF) or 20 \(\mu\)L
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 minute and the
layers were allowed to separate for 16-24 hours. 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 Equation 2:

\[
\text{Energy transfer efficiency} = \frac{I_{DA}}{I_A} \times 100\%
\]  

(Eq. 2)

where \(I_{DA}\) is the integration of the fluorophore emission from analyte excitation and \(I_A\) is the
integrated fluorophore emission from direct excitation.

**RESULTS AND DISCUSSION**
PAHs found in oil collected from environmental oil spills have undergone substantial oxidation to a variety of highly polar, oxidized products, including quinones, phenols, and other oxidized species. Consistent with these reports, when the oil spill oil was mixed with an aqueous buffer solution (0 mM cyclodextrin), it demonstrated a high concentration of photophysically active compounds partitioning into the aqueous buffer solution (Figure 1B). Water soluble photophysically active compounds extracted from oils are likely to be oxidized PAH metabolites or other water soluble aromatic moieties, a hypothesis that is supported by ample literature precedent. In contrast, only a negligible concentration of photophysically active compounds partitioned from the motor oil into a cyclodextrin-free aqueous layer, reflecting the lower degree of polar fluorescent metabolites found in that oil (Figure 1A). The oil-water partitioning of tar balls was intermediate between the oil spill oil and the motor oil, with 46% of the overall fluorescence found in the aqueous buffer layer (Figure 1C). The differential behavior of tar balls compared to oil spill oil can be explained by the different composition of the tar balls – they are enriched in heavier components, such as asphaltenes, that are insoluble in water. The PAHs found in the tar ball’s interior are also somewhat protected from oxidation due to their limited interaction with the oxygen-rich environment, whereas the PAHs in oil spill oil are more susceptible to oxidation.
Figure 1. Analyte comparisons in buffer-oil mixtures for (A) motor oil; (B) oil spill oil; and (C) tar ball oil. The black line represents fluorescence of the aqueous layer and the grey line represents fluorescence of the oil layer. All samples were excited at 360 nm.

The addition of cyclodextrin to the aqueous solutions has the potential to alter this partitioning between the aqueous and oil layers, because cyclodextrins have been shown to bind PAHs and other aromatic analytes with high efficiencies.\textsuperscript{40,41} For the motor oil-buffer solutions, the addition of $\gamma$-cyclodextrin and 2-HPCD led to a substantial increase in the amount of photophysically active compounds extracted into the aqueous layer (from 24.0\% in PBS to 33.6\% and 34\% for 2-HPCD and $\gamma$-cyclodextrin respectively), which is consistent with the known ability of these cyclodextrins to bind PAHs. Other cyclodextrin derivatives, including $\beta$-cyclodextrin, methyl-$\beta$-cyclodextrin, and $\alpha$-cyclodextrin, have cavity sizes that are too small to bind many PAHs, and their addition had no effect on the oil-water fluorescence ratios (Table 1).

### Table 1. Percentage of analyte found in the aqueous layer of oil-buffer solutions with a variety of cyclodextrin derivatives\textsuperscript{a}

| Cyclodextrin derivative | Motor oil | Oil spill oil | Tar ball oil |
|-------------------------|-----------|---------------|--------------|
| PBS                     | 24.0\%    | 67.2\%        | 46.8\%       |
| $\gamma$-cyclodextrin   | 5.9\%     | 59.4\%        | 48.6\%       |
| $\beta$-cyclodextrin    | 10.3\%    | 71.9\%        | 44.6\%       |
| Me-$\beta$-cyclodextrin | 4.7\%     | 71.7\%        | 69.3\%       |
| 2-HPCD                  | 33.6\%    | 37.2\%        | 65.2\%       |
| $\gamma$-cyclodextrin   | 33.4\%    | 50.9\%        | 53.7\%       |

\textsuperscript{a} All analyte comparisons were calculated using Equation 1, with undoped oil samples. All reported results represent an average of at least 3 trials.

For the oil spill oil-aqueous mixtures, the addition of both 2-HPCD and $\gamma$-cyclodextrin increased the fluorescence of both the oil layer and the aqueous layer. However, the fluorescence
of the oil layer increased to a much greater extent (6.95-fold) compared to that of the aqueous layer (2.42-fold increase) (Figure 2), leading to an overall decrease in the percentage of fluorescently active compounds found in the aqueous layer. These fluorescence increases can be explained by the cyclodextrin binding a variety of PAHs and PAH metabolites in both the aqueous and oil phases; in each case, binding of the fluorescent small molecules leads to a noticeable fluorescence increase through the elimination of non-radiative decay pathways.\(^{42}\)

![Graph showing fluorescence changes](image)

**Figure 2.** Changes in the fluorescence in oil spill oil-buffer solutions with the addition of various cyclodextrins in the (A) aqueous layer; and (B) oil layer. The black line shows the fluorescence in a PBS-oil solution (no cyclodextrin), the blue line shows the fluorescence in a \(\gamma\)-cyclodextrin-oil solution, and the red line shows the fluorescence in a 2-HPCD-oil solution. These results are representative results of 3 independent trials. *(Color figure requested online and in print)*

For the tar ball oil-buffer mixtures, the addition of all cyclodextrin derivatives led to modest enhancements in the fluorescence ratios of the aqueous layer, with the exception of \(\beta\)-cyclodextrin which showed no change in the extraction efficiencies. These results indicate that the cyclodextrins are moderately effective in extracting photophysically active analytes from the crude tar ball solution. The larger cyclodextrins likely extract PAHs via hydrophobic encapsulation of the hydrophobic PAHs, analogous to what is observed with motor oil samples.
and what we reported in our previous publication.\textsuperscript{16} However, the addition of the smaller cyclodextrins also led to an increase in the percentage of fluorescence found in the aqueous layer, even though such cyclodextrins lack sufficient steric bulk to encapsulate PAHs in their hydrophobic cavities. These cyclodextrins are likely effecting fluorescence increases by binding polar PAH analytes via hydrogen bond formation;\textsuperscript{43} this hydrogen bonding allows analytes that are too large to bind in the cyclodextrin interior to associate with the cyclodextrins, thereby enabling enhanced extraction into the aqueous layer.

Following the efficient extraction of PAHs from a variety of complex oils using cyclodextrin derivatives, the ability of the newly extracted PAHs to participate in cyclodextrin-promoted energy transfer in the aqueous layer was assayed. This energy transfer requires that fluorophore 4 partition efficiently into the aqueous layer. The percentage of fluorophore emission in the aqueous layer was measured for all oil-cyclodextrin combinations, and found to be particularly efficient for methyl-\(\beta\)-cyclodextrin containing solutions (Figure 3). This high efficiency points to a high degree of steric and electronic compatibility between methyl-\(\beta\)-cyclodextrin and fluorophore 4. Notably, some degree of fluorescence emission from fluorophore 4 was found in the aqueous layer for all oil-cyclodextrin combinations, indicating the potential for efficient energy transfer in all cases.
Figure 3. Fluorophore 4 emission in aqueous-oil mixtures for (A) motor oil; (B) oil spill oil; and (C) tar ball oil. The black line represents fluorescence of the aqueous layer without cyclodextrin and the grey line represents fluorescence of the aqueous layer with 10 mM of methyl-cyclodextrin. All samples were excited at 460 nm.

Energy transfer in the aqueous layer was measured for all cyclodextrin-oil combinations, and some key results are summarized in Tables 2-5.

Table 2. Energy transfer efficiencies in the undoped aqueous extracts

| Cyclodextrin derivative | Motor oil | Oil spill oil | Tar ball oil |
|------------------------|-----------|---------------|--------------|
| PBS                    | $b$        | 50.0%         | 23.9%        |
| -cyclodextrin          | 36.8%     | 51.8%         | 33.3%        |
| -cyclodextrin          | 45.9%     | 29.5%         | 20.4%        |
| Me- -cyclodextrin      | 35.9%     | 24.1%         | 31.6%        |
| 2-HPCD                 | 74.4%     | 85.7%         | 34.5%        |
| -cyclodextrin          | 73.0%     | 86.4%         | 28.5%        |

*a All values represent an average of at least 3 trials

*b No energy transfer peak was observed

Table 3. Energy transfer efficiencies in the aqueous extracts doped with analyte 1
| Cyclodextrin derivative | Motor oil | Oil spill oil | Tar ball oil |
|------------------------|-----------|---------------|--------------|
| PBS                    | 9.0%      | 78.7%         | 24.8%        |
| -cyclodextrin          | \textit{b} | 30.2%         | 32.5%        |
| -cyclodextrin          | 46.2%     | 34.4%         | 23.3%        |
| Me- -cyclodextrin      | 38.7%     | 26.1%         | 29.5%        |
| 2-HPCD                 | \textit{b} | 80.1%         | 26.6%        |
| -cyclodextrin          | 71.0%     | 77.2%         | 28.1%        |

\textit{a} All values represent an average of at least 3 trials

\textit{b} No energy transfer peak was observed

\textbf{Table 4.} Energy transfer efficiencies in the aqueous extracts doped with analyte 2\textsuperscript{a}

| Cyclodextrin derivative | Motor oil | Oil spill oil | Tar ball oil |
|------------------------|-----------|---------------|--------------|
| PBS                    | 80.5%     | 68.8%         | 26.2%        |
| -cyclodextrin          | 57.7%     | 28.3%         | 32.9%        |
| -cyclodextrin          | 49.2%     | 34.2%         | 23.6%        |
| Me- -cyclodextrin      | 38.1%     | 28.2%         | 29.7%        |
| 2-HPCD                 | 85.4%     | 73.8%         | 27.1%        |
| -cyclodextrin          | 71.0%     | 80.1%         | 29.3%        |

\textit{a} All values represent an average of at least 3 trials

\textbf{Table 5.} Energy transfer efficiencies in the aqueous extracts doped with analyte 3\textsuperscript{a}
For oil spill oil, the observed energy transfer efficiency with undoped samples in the absence of any cyclodextrin was fairly high, and the addition of β-cyclodextrin and methyl-β-cyclodextrin led to decreases in the observed energy transfer efficiencies (energy transfer efficiencies of 30% and 24% for β-cyclodextrin and methyl-β-cyclodextrin, respectively, compared to 50% in the absence of any cyclodextrin) (Table 2). The addition of larger cyclodextrins (i.e. 2-HPCD and γ-cyclodextrin) caused a substantial enhancement in the observed affinities. The large degree of cyclodextrin-free energy transfer is consistent with our previously reported results that showed cyclodextrin-free association in many complex environments. In these aqueous extracts, PAH metabolites likely associate with fluorophore 4 via a combination of hydrophobic binding (between the aromatic portions of the metabolites and the aromatic moieties of the fluorophore) and hydrogen bonding (between the hydroxyl and carbonyl moieties of the metabolites and the thiol and charged portions of the fluorophore); this close association is responsible for the observed cyclodextrin-free energy transfer.

For oil collected from tar balls, a modest energy transfer efficiency in the cyclodextrin-free solution was observed in undoped samples, and this efficiency was somewhat enhanced by the addition of most cyclodextrin derivatives by 8-10 percentage points (Table 2), with only β-cyclodextrin leading to a slight decrease in the energy transfer efficiencies. The most likely explanation for this scenario is that cyclodextrins facilitate the association of the aromatic toxicants with fluorophore 4. This facilitated association can either occur via the formation of a ternary complex in the cyclodextrin cavity (as has been demonstrated for α-cyclodextrin).
and 2-HPCD$^{46,47}$, or via association of one of the two energy transfer partners outside the cyclodextrin cavity (a more likely scenario for the smaller cyclodextrin derivatives).

In aqueous extracts from motor oil, the degree of cyclodextrin-free energy transfer varied depending on the identity of the doped analyte, with analytes 2 and 3 demonstrating substantially higher degrees of cyclodextrin-free energy transfer compared to analyte 1. This is consistent with our previously reported results that demonstrated that analytes with large hydrophobic surface areas are most likely to engage in cyclodextrin-free association and cyclodextrin-independent energy transfer.$^{19}$ The energy transfer efficiencies were most improved by the addition of 2-HPCD and $\gamma$-cyclodextrin, with 73% and 74% efficiencies observed using $\gamma$-cyclodextrin and 2-HPCD, respectively. These results are consistent with the known ability of these cyclodextrins to form ternary complexes that promote proximity-induced energy transfer.$^{48}$

The results in Table 2 highlight the ability of cyclodextrin to remove aromatic toxicants from both oil spill oil and tar ball oil. These experiments, conducted without doping a particular PAH into the complex mixture, involve the cyclodextrins extracting a wide range of toxicants from the complex oils, including PAHs, PAH metabolites, and other aromatic moieties. Overall, the results reported herein highlight the potential of cyclodextrin derivatives to promote the efficient extraction of small-molecule toxicants from oil spills, as well as their subsequent detection via energy transfer to a high quantum yield fluorophore. This system has a number of notable advantages, including:

1. In contrast to our previously reported results that demonstrated modest extraction efficiencies using $\gamma$-cyclodextrin to extract PAHs from motor oil, vegetable oil, and vacuum pump oil, we report herein substantially improved extraction efficiencies using a variety of cyclodextrin derivatives to extract aromatic toxicants from oil spill oil and tar ball oil, with up to 72% of the aromatic toxicants found in the cyclodextrin-containing aqueous layer, compared to
our previously reported best of 34% aromatic analytes in \( \gamma \)-cyclodextrin-containing aqueous layer extracted from motor oil. Oil collected directly from oil spill sites and oil isolated from tar balls have different physicochemical profiles compared to motor oil, vegetable oil, and vacuum pump oil, as a result of the weathering process that promotes substantial oxidation of the aromatic toxicants.\(^5\) Environmental remediation of oil spill oil and tar ball oil from polluted marine environments is substantially more relevant for environmental disaster efforts than the remediation of commercially available oils, and the results reported herein indicate that using a variety of cyclodextrin derivatives enables the efficient extraction of toxicants from these complex oils.

(2) The cyclodextrin-based extraction followed by detection system reported herein provides a rapid method to remove toxicants from oil spills and to confirm that photophysically active analytes were removed via fluorescence energy transfer, which is a useful tool in disaster response efforts. In many oil spill situations, the precise identification of each toxicant is less crucial than the ability to remove as many toxicants as possible as quickly as possible and confirm such removal. Using cyclodextrin derivatives to enhance the extraction of photophysically active compounds from the oil layer to the aqueous layer, as demonstrated herein, provides a practical method for such environmental detoxification, and monitoring the overall fluorescence of the extracted analytes provides a rapid method to assay the efficacy of such detoxification procedures.

CONCLUSION

In conclusion, the results reported herein demonstrate that cyclodextrin-based systems can be used for the efficient extraction and detection of aromatic toxicants from real-world oil samples collected at the sites of oil spills. The system uses a number of commercially-available, non-toxic cyclodextrin derivatives to optimize extraction and detection procedures for each oil sample
investigated, and demonstrate that our previously-reported results are generally applicable for the cleanup of oil-contaminated marine environments. These results also pointed to the potential of using multiple cyclodextrins simultaneously for the cleanup of a single oil system, with the cyclodextrins that are optimal for extraction of PAHs, binding of the fluorophore, and promotion of efficient energy transfer combined into a single high-performing, multi-cyclodextrin system. Research in this direction is currently underway in our group, and the results to date support this idea. The full results will be reported in due course.

ASSOCIATED CONTENT

Supporting Information. Detailed experimental procedures, synthetic procedures for the synthesis of fluorophore 4, all summary tables and summary figures for all analyte-oil-cyclodextrin combinations. This material is available free of charge via the Internet.

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Author Contributions

The manuscript was written through contributions of all authors, and all authors have given approval to the final version of the manuscript.

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