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C60 reduces the bioavailability of mercury in aqueous solutions

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Abstract
The effects of C60 on mercury bioavailability and sorption were investigated at different C60 dosages, reaction times, and pH ranges using the merR::luxCDABE bioluminescent bioreporter Escherichia coli AR1I. The results demonstrated that the bioavailability of mercury (Hg\(^{2+}\)) decreased with increasing C60 dosage. Approximately 30% of aqueous mercury became biologically unavailable 2 h after interaction with C60 at a mass ratio of C60 to mercury as low as 0.01. However, this reduction in bioavailability plateaued at a mass ratio of C60 to mercury of 10 with a further increase in C60 concentrations resulting in only a 20% additional decrease in bioavailability. If this reduction in bioluminescence output is attributable to mercury sorption on C60, then each one log-order increase in C60 concentration resulted in a 0.86 log-order decrease in the mercury partitioning coefficient (K\(d\)). This relationship implies the presence of high mercury-affinitive sites on C60. The length of reaction time was found to play a more important role than C60 dosage in reducing Hg\(^{2+}\) bioavailability, suggesting an overall slow kinetics of the C60–Hg interactions. In addition, lowering the pH from 7.2 to 5.8 decreased mercury bioavailability due likely to the increase in mercury's association with C60. These results suggest that C60 may be useful in capturing soluble mercury and thus reducing mercury biotoxicity.

1. Introduction
Maintaining a sufficient supply of clean water for drinking is critical for society, particularly in geographic locations with rapid population growth and urbanization (Reiter et al., 2004). However, contamination of water supply sources continues to be problematic, with heavy metals being of particular concern. Mercury, for example, is highly toxic towards biological systems and, once absorbed by microbiota, becomes bioaccumulated in the food chain with consequent toxic impacts to higher order animal and human health. Due to mercury's elevated toxicity, stringent regulatory controls have been imposed on mercury concentrations in the environment. For instance, mercury is a priority pollutant that the U.S. Environmental Protection Agency has set a maximum contaminant level of 2 \(\mu\)g L\(^{-1}\) in drinking water (USEPA, 2009).

To protect drinking water, inexpensive and efficient methods for monitoring and removing mercury from natural water and soil runoff are of great interest. Conventional technologies, such as membrane filtration, ion exchange, solvent extraction, and biological degradation, are reasonably efficient for mercury removal (Jones and Slotton, 1996; Nansu-Njiki et al., 2009; USEPA, 1997), but are relatively costly and time-consuming. Consequently, the search for new and advanced technologies for mercury removal continues. Nanotechnology, using materials and structures with
nanscale dimensions of 1–100 nm, is a promising technology widely studied for water treatment (Colvin, 2003; Masciandri and Zhang, 2003; Savage and Diallo, 2005). For example, the suitability of nanocarbon and metal oxides in removing different types of water contaminants has been well investigated in the last decade (Meyyappan and Srivastava, 2000). Previous studies mainly focused on nanocarbon tubes or modified traditional sorbents (e.g., active carbon) (Cooper et al., 2010; Tawabini et al., 2010) and indicate that the sorption capacity of nanomaterials for metals primarily depends on their nanosize and surface area.

Theoretically, C60 fullerenes should have an adsorption capacity similar to carbon nanotubes due to their high hydrophobic surface area and large inner volume of spherical structures. Pyrzynska (2007) reported that C60 could extract low polar metal complexes from aqueous solution through r-electron interactions between them, and that maximum chelate adsorption was achieved within a pH range of 0.5–5.0. C60 was also found to exhibit better sorption properties for metal than conventional reversed-phase sorbent materials such as silica-bonded C18. Lesniewska et al. (2005) reported that the neutral chelates of lead, cadmium, and palladium formed with ammonium pyrrolidinedithiocarbamate or diethyldithiocarbamate could be sorbed onto a microcolumn packed with C60. Yoon et al. (2008) found that calcium and strontium could be strongly bound to C60 through charge transfer mechanisms. This charge redistribution, in turn, gives rise to electric fields surrounding the coated C60. Recent studies have suggested that lithium adsorption preferably occurs on C60 in a carbon hybrid material system consisting of single-wall carbon nanotubes (SWCNTs) and C60 due to larger adsorption energy on C60 than on pure SWCNTs (Koh et al., 2011). All of these studies provide insights into the potential of C60 for mercury removal. However, the metal affinity, indicated by the partitioning coefficient (i.e., a loss of bioluminescent light from this bioreporter indicated i.e., a loss of bioluminescent light from this bioreporter indicated a decrease in light output), of C60 towards mercury bioavailability, mixtures of mercury at concentrations (Cm)– and mercury or C60 toxicity (i.e., a loss of bioluminescent light from this bioreporter indicated mercury or C60 concentrations toxic to E. coli).

2.2. Bioluminescent bioavailability assay for mercury

Bioavailable mercury was measured as a function of the bioluminescence emission intensity of the merR::luxCDABE-based bioreporter E. coli ARL1 using a BioTek Synergy multi-mode microplate reader (Winooski, VT, USA). E. coli ARL1 harbors a chromosomally inserted ~500 bp region of the mer operon consisting of the merK gene and the promoter/operator region of the mer operon fused to the luxCDABE gene cassette of Photobacterium phosphoreum (Dahl et al., 2011). Its detection limit for bioavailable Hg (II) is approximately 2 μg L⁻¹ and it effectively differentiates between Hg²⁺ and Hg-EDTA complex (Dahl et al., 2011). Bioluminescent assays were performed by growing the ARL1 bioreporter from ~80 °C freezer stock in 25 mL Luria–Bertani (LB) broth supplemented with kanamycin at 50 mg L⁻¹ (Kₐₙₐ₃) at 30 °C with shaking (200 rpm) to an optical density at 600 nm (OD₆₀₀) of 0.7. The culture was then diluted 1:10 in fresh LB broth + Kₐₙₐ₃ and incubated with shaking (200 rpm) at 30 °C to an OD₆₀₀ of 0.30 (~1 × 10⁶ cfu mL⁻¹, exponential growth phase). Aliquots of 900 μL of the culture were then transferred to black 24-well microtiter plate wells (Corning, NY, USA) containing 100 μL of the HgCl₂/C60 dilutions as explained above. At least three replicates of each 24-well plate were performed and a minimum of three replicates for each dilution were included in each plate. Plates were sealed with a transparent membrane (Breathe-Easy, Sigma–Aldrich, St. Louis, MO, USA) and placed in the BioTek Synergy plate reader for bioluminescence detection at 28 °C. Bioluminescence in counts per second (cps) was recorded with a 1 s integration time every 5 min for 2 h. Each plate contained triplicate controls consisting of E. coli ARL1 + HgCl₂ at 1 μg mL⁻¹. E. coli ARL1 alone, E. coli ARL1 + 100 μL toluene, and E. coli ARL1 + 500 μg C60. An additional constitutively bioluminescent bioreporter, E. coli 652T7, consisting of a T7 promoter fusion to the P. luminescens luxCDABE gene cassette, was included in triplicate in each plate as a control to establish mercury or C60 toxicity (i.e., a loss of bioluminescent light from this bioreporter indicated mercury or C60 concentrations toxic to E. coli).

2.3. Experiments establishing C60 exposure time effects

For experiments demonstrating the effect of exposure time of C60 towards mercury bioavailability, mixtures of mercury at 0.74 μg mL⁻¹ in deionized water were combined with C60 at dosages of 0.01, 0.05, 0.1, 0.5, or 1.0 μg and incubated at room temperature with shaking (200 rpm) for either 30 min or 120 min, after which aliquots were removed and transferred to 24-well microtiter plates for the determination of bioavailability as described above.

2.4. Data analysis

The amount of mercury adsorbed on C60 was determined by calculating the difference between the initial (C₁) and equilibrium concentrations (C_m). The mercury removal (%) from the solution was calculated as:

\[ R = \frac{C_1 - C_m}{C_1} \times 100 \]  

(1)

The metal affinity, indicated by the partitioning coefficient K_d (μg mL⁻¹), was calculated as:

\[ K_d = \frac{C_1 - C_m}{M_a} \times V \]  

(2)

where V is the solution volume (mL) and M_a is the mass of C60 (μg).

All tests were performed in at least triplicate and results expressed as the mean ± standard deviation. Significance was established using an analysis of variance (ANOVA) at p < 0.05.
3. Results and discussion

3.1. Effect of C60 dosage

The effect of C60 on mercury bioavailability was first investigated using dosages of C60 between 1 and 500 \text{lg} (corresponding to mass ratios of C60 to Hg\textsuperscript{2+} from 1.35 to 675) under a constant Hg\textsuperscript{2+} exposure concentration of 0.74 \text{lgm L}/C0. \textsuperscript{1} The results demonstrated that mercury bioavailability, as measured by the bioluminescent bioreporter \textit{E. coli} ARL1, significantly decreased in the presence of C60 and was dose dependent across the 1, 10, 100, and 500 \text{lg} C60 dosages tested (Fig. 1). No significant bioluminescence was detected among the controls (\textit{E. coli} ARL1 alone and \textit{E. coli} ARL1 + C60 at 500 \text{ug}) nor did \textit{E. coli} ARL1 respond to residual levels of toluene potentially remaining after the C60 preparation. Bioluminescence from the constitutive control bioreporter \textit{E. coli} 652T7 was not significantly affected by C60 or mercury within the experimental concentrations applied, thus demonstrating that C60 and mercury were not themselves toxic towards \textit{E. coli} under our experimental conditions (data not shown).

The \textit{E. coli} ARL1 bioreporter bioluminescence emission profiles were used to indicate Hg\textsuperscript{2+} bioavailability and demonstrated that Hg\textsuperscript{2+} bioavailability decreased with increasing C60 concentrations (Fig. 2a). The addition of a very small amount of C60 (e.g., 1% of Hg\textsuperscript{2+} mass) reduced Hg\textsuperscript{2+} bioavailability by 30%. However, a further five log-order increase in C60 concentration resulted only in an additional 20% decrease in bioavailability. If the decrease in bioluminescence from \textit{E. coli} ARL1 is attributed to Hg\textsuperscript{2+} adsorption on C60, the partitioning coefficients (K\textsubscript{d}) of Hg\textsuperscript{2+} on C60 decreased with increasing C60 dosage (Fig. 2b). The fitted equation indicated that each one log-order increase in C60 concentration resulted in a decrease in the K\textsubscript{d} value by an order of 0.86. This moderate inconsistency implies the presence of sorption sites having high affinity for Hg\textsuperscript{2+}. Our calculations indicate that these high-affinity sites may account for approximately 14% of the total sorption sites, with the majority of sites being relatively uniform in affinity for Hg\textsuperscript{2+}. This variability in affinity, however, requires further study through chemical characterization.

3.2. Effect of C60 exposure time on mercury bioavailability

Fig. 3 shows the reduction of bioavailable mercury starting at a concentration of 0.74 \text{ugm mL}^{-1} after exposure to C60 for 30 min versus 120 min at five low C60 dosages (0.01, 0.05, 0.1, 0.5, and 1 \text{ug}). These dosages are equivalent to the mass ratios of C60 to Hg\textsuperscript{2+} of 0.014, 0.068, 0.135, 0.675, and 1.354, respectively. The longer exposure time of 120 min reduced mercury bioavailability significantly compared to the 30 min exposure time. The 30 min exposure resulted in less than 5% of the mercury becoming unavailable to the \textit{E. coli} ARL1 bioreporter bacteria, whereas the 120 min exposure resulted in 30–40% of the mercury to becoming unavailable at the experimental dosages. This time dependent reduction in bioavailability is likely due to sorption of Hg\textsuperscript{2+} by C60 since \textit{E. coli} ARL1 responds only to ionic mercury (Hg\textsuperscript{2+}). Once associated with C60, Hg\textsuperscript{2+} is unable to be taken up by \textit{E. coli} ARL1, thus resulting in the reduced bioluminescent response.

3.3. Effect of pH on mercury bioavailability

Fig. 4 shows the effect of pH on the bioluminescence emission profile of \textit{E. coli} ARL1 during interaction with Hg\textsuperscript{2+} at a starting concentration of 0.74 \text{ugm mL}^{-1}. Lowering the pH from 7.2 to 5.8 reduced bioluminescence emission over a wide range of mass ratios of C60 to Hg\textsuperscript{2+} (0.01–1.35). This reduction in bioluminescence could be attributed to a decrease in Hg\textsuperscript{2+} uptake by strain ARL1 due to pH-induced alteration of bacterial physiology and/or Hg\textsuperscript{2+} sorption on C60. Previous studies have shown that acidification increases intracellular accumulation of Hg\textsuperscript{2+} and enhances

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Fig. 1. Bioluminescence emission profile of the \textit{E. coli} ARL1 bioreporter during a 2 h exposure to C60 at dosages ranging from 1 to 500 \text{ug} in the presence of a constant 0.74 \text{ugm mL}^{-1} concentration of Hg\textsuperscript{2+}. cps: bioluminescent counts/s.

Fig. 2. Effect of C60 on (a) mercury bioavailability and (b) mercury partition coefficient (K\textsubscript{d}) after a 2 h exposure at a constant 0.74 \text{ugm mL}^{-1} concentration of Hg\textsuperscript{2+}.
light emission (Golding et al., 2002; 2008; Kelly et al., 2003). Therefore, the opposite pH effect observed in this study was dominantly caused by enhanced association of Hg\(^{2+}\) on C60 at lower pH. As demonstrated by the differences in bioluminescence emission intensity, the pH effect was reduced as C60 dosage increased. This is in accordance with Schindler et al. (1980) who showed that mercury ions bind to organic matter more strongly as solution pH decreases. Tawabini et al. (2010) observed a negligible effect of solution pH on mercury sorption by multiwalled carbon nanotubes (MWCNTs) between pH 5 and 7 at high mass ratios of carbon to mercury (1000–2000:1). However, they reported that mercury removal increased from 73% at pH 4 to 93% at pH 5 and decreased from 95% at pH 8 to 81% at pH 9. They attributed the varying effects at different ranges of pH values to the changes in the surface charge of MWCNTs and the composition of the aqueous mercury species. Our results suggest that the concentration of carbon nanoparticles is also a factor that influences the pH effect. For example, the pH effect was significantly smaller at the C60:Hg mass ratio of 1.35 than at 0.01 (Fig. 4d versus a). This trend corresponds with the minimal pH effect observed by Tawabini et al. (2010) at high MWCNT dosages (e.g., MWCNT:Hg mass ratios of 1000–2000) in the same range of pH values (i.e., 5–7). Our results indicate that a pH rendered change in surface charge exerts limited effects on mercury association with C60. One of the main mechanisms involved is that of acidification decreasing the concentrations of negative mercury species (e.g., Hg(OH)\(_{3}\)\(^{-}\) and Hg(OH)\(_{4}\)\(^{2-}\)) while increasing the concentration of Hg\(^{2+}\). The latter positively species exhibits stronger adsorption on negatively charged C60 than the former two negatively charged species. As a result, mercury is more strongly bound to C60 at lower pH, leading to decreased bioavailability of mercury to the bioreporter and, thus, lower bioluminescence emission. However, mercury that is associated with environmental media (e.g., colloids) can become bioavailable under certain pH conditions. Such pH-mediated bioavailability would be expected to lead to mercury accumulation in higher organisms (e.g., fish) in surface water with bulk mercury contamination (Jones and Slotton, 1996). Nonetheless, the complicated relationships between solution pH, mercury species, sorption, and bioavailability require further study.

4. Conclusions

Based on the bioavailability profiles of the E. coli ARL1 bioluminescent bioreporter, it was demonstrated that C60 is efficient in reducing the bioavailability of Hg\(^{2+}\) through association primarily mediated by sorption. The sorption efficiency varies with C60 dosage, reaction time, and pH. Specific findings of this study are:
• Increases in C60 dosage reduce mercury bioavailability. Thirty percent of Hg²⁺ became unavailable to the ARL1 bioreporter bacteria when the mass ratio of C60 to Hg²⁺ was 0.01.
• The length of reaction time plays a more important role than C60 dosage in reducing Hg²⁺ bioavailability, suggesting slow kinetics of the C60–Hg interactions.
• Lowering the solution pH from 7.2 to 5.8 decreases mercury bioavailability, and this effect is more significant at lower C60 dosages.

Overall, this study provides meaningful insights into the potential of C60 in reducing the biotoxicity of mercury in the environment. The low mass density of C60 makes it easy to disperse in aqueous environments, under appropriate risk assessment controls, especially in wastewater treatment plants and constructed wetlands where water remains relatively stagnant and is susceptible to substantial metal pollution. The large inner surface area of C60 and its strong association with mercury make it a promising candidate for improving existing filtration materials (e.g., activated carbon). Furthermore, the reduction in bioluminescence exhibited by E. coli ARL1 in the presence of C60, particularly under acidic conditions, provides insights into the potential of C60 for influencing the overall biotransformation of mercury in the environment. The association of C60 with mercury may also inhibit the cytotoxic effect of C60 on biological cells. Nonetheless, clarification of these issues need further investigation with consideration of natural environmental factors, such as dissolved organic matter and other metal ions.

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References